illumina®

HiSeq® 1000 System User Guide

FOR RESEARCH USE ONLY

ILLUMINA PROPRIETARY

Part # 15023355 Rev. L
November 2013

This document and its contents are proprietary to Illumina, Inc. and its affiliates ("Illumina"), and are intended solely for the contractual use of its customer in connection with the use of the product(s) described herein and for no other purpose. This document and its contents shall not be used or distributed for any other purpose and/or otherwise communicated, disclosed, or reproduced in any way whatsoever without the prior written consent of Illumina. Illumina does not convey any license under its patent, trademark, copyright, or common-law rights nor similar rights of any third parties by this document.

The Software is licensed to you under the terms and conditions of the Illumina Sequencing Software License Agreement in a separate document. If you do not agree to the terms and conditions therein, Illumina does not license the Software to you, and you should not use or install the Software

The instructions in this document must be strictly and explicitly followed by qualified and properly trained personnel in order to ensure the proper and safe use of the product(s) described herein. All of the contents of this document must be fully read and understood prior to using such product (s).

FAILURE TO COMPLETELY READ AND EXPLICITLY FOLLOW ALL OF THE INSTRUCTIONS CONTAINED HEREIN MAY RESULT IN DAMAGE TO THE PRODUCT(S), INJURY TO PERSONS, INCLUDING TO USERS OR OTHERS, AND DAMAGE TO OTHER PROPERTY.

ILLUMINA DOES NOT ASSUME ANY LIABILITY ARISING OUT OF THE IMPROPER USE OF THE PRODUCT(S) DESCRIBED HEREIN (INCLUDING PARTS THEREOF OR SOFTWARE) OR ANY USE OF SUCH PRODUCT(S) OUTSIDE THE SCOPE OF THE EXPRESS WRITTEN LICENSES OR PERMISSIONS GRANTED BY ILLUMINA IN CONNECTION WITH CUSTOMER'S ACQUISITION OF SUCH PRODUCT(S).

FOR RESEARCH USE ONLY

© 2013 Illumina, Inc. All rights reserved.

Illumina, **IlluminaDx**, **BaseSpace**, **BeadArray**, **BeadXpress**, **cBot**, **CSPro**, **DASL**, **DesignStudio**, **Eco**, **GAIIx**, **Genetic Energy**, **Genome Analyzer**, **GenomeStudio**, **GoldenGate**, **HiScan**, **HiSeq**, **Infinium**, **iSelect**, **MiSeq**, **Nextera**, **NuPCR**, **SeqMonitor**, **Solexa**, **TruSeq**, **TruSight**, **VeraCode**, the pumpkin orange color, and the Genetic Energy streaming bases design are trademarks or registered trademarks of Illumina, Inc. All other brands and names contained herein are the property of their respective owners.

This software contains the SeqAn Library, which is licensed to Illumina and distributed under the following license:

Copyright © 2010, Knut Reinert, FU Berlin, All rights reserved. Redistribution and use in source and binary forms, with or without modification, are permitted provided that the following conditions are met:

1 Redistributions of source code must retain the above copyright notice, this list of conditions and the following disclaimer.

2 Redistributions in binary form must reproduce the above copyright notice, this list of conditions and the following disclaimer in the documentation and/or other materials provided with the distribution.

3 Neither the name of the FU Berlin or Knut Reinert nor the names of its contributors may be used to endorse or promote products derived from this software without specific prior written permission.

THIS SOFTWARE IS PROVIDED BY THE COPYRIGHT HOLDERS AND CONTRIBUTORS "AS IS" AND ANY EXPRESS OR IMPLIED WARRANTIES, INCLUDING, BUT NOT LIMITED TO, THE IMPLIED WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE ARE DISCLAIMED. IN NO EVENT SHALL THE COPYRIGHT HOLDER OR CONTRIBUTORS BE LIABLE FOR ANY DIRECT, INDIRECT, INCIDENTAL, SPECIAL, EXEMPLARY, OR CONSEQUENTIAL DAMAGES (INCLUDING, BUT NOT LIMITED TO, PROCUREMENT OF SUBSTITUTE GOODS OR SERVICES; LOSS OF USE, DATA, OR PROFITS; OR BUSINESS INTERRUPTION) HOWEVER CAUSED AND ON ANY THEORY OF LIABILITY, WHETHER IN CONTRACT, STRICT LIABILITY, OR TORT (INCLUDING NEGLIGENCE OR OTHERWISE) ARISING IN ANY WAY OUT OF THE USE OF THIS SOFTWARE, EVEN IF ADVISED OF THE POSSIBILITY OF SUCH DAMAGE.

Read Before Using this Product

This Product, and its use and disposition, is subject to the following terms and conditions. If Purchaser does not agree to these terms and conditions then Purchaser is not authorized by Illumina to use this Product and Purchaser must not use this Product.

1 **Definitions**. "**Application Specific IP**" means Illumina owned or controlled intellectual property rights that pertain to this Product (and use thereof) only with regard to specific field(s) or specific application(s). Application Specific IP excludes all Illumina owned or controlled intellectual property that cover aspects or features of this Product (or use thereof) that are common to this Product in all possible applications and all possible fields of use (the "**Core IP**"). Application Specific IP and Core IP are separate, non-overlapping, subsets of all Illumina owned or controlled intellectual property. By way of non-limiting example, Illumina intellectual property rights for specific diagnostic methods, for specific forensic methods, or for specific nucleic acid biomarkers, sequences, or combinations of biomarkers or sequences are examples of Application Specific IP. "**Consumable(s)**" means Illumina branded reagents and consumable items that are intended by Illumina for use with, and are to be consumed through the use of, Hardware. "**Documentation**" means Illumina's user manual for this Product, including without limitation, package inserts, and any other documentation that accompany this Product or that are referenced by the Product or in the packaging for the Product in effect on the date of shipment from Illumina. Documentation includes this document. "**Hardware**" means Illumina branded instruments, accessories or peripherals. "**Illumina**" means Illumina, Inc. or an Illumina affiliate, as applicable. "**Product**" means the product that this document accompanies (e.g., Hardware, Consumables, or Software). "**Purchaser**" is the person or entity that rightfully and legally acquires this Product from Illumina or an Illumina authorized dealer. "**Software**" means Illumina branded software (e.g., Hardware operating software, data analysis software). All Software is licensed and not sold and may be subject to additional terms found in the Software's end user license agreement. "**Specifications**" means Illumina's written specifications for this Product in effect on the date that the Product ships from Illumina.

2 **Research Use Only Rights**. Subject to these terms and conditions and unless otherwise agreed upon in writing by an officer of Illumina, Purchaser is granted only a non-exclusive, non-transferable, personal, non-sublicensable right under Illumina's Core IP, in existence on the date that this Product ships from Illumina, solely to use this Product in Purchaser's facility for Purchaser's internal research purposes (which includes research services provided to third parties) and solely in accordance with this Product's Documentation, **but specifically excluding any use that** (a) would require rights or a license from Illumina to Application Specific IP, (b) is a re-use of a previously used Consumable, (c) is the disassembling, reverse-engineering, reverse-compiling, or reverse-assembling of this Product, (d) is the separation, extraction, or isolation of components of this Product or other unauthorized analysis of this Product, (e) gains access to or determines the methods of operation of this Product, (f) is the use of non-Illumina reagent/consumables with Illumina's Hardware (does not apply if the Specifications or Documentation state otherwise), or (g) is the transfer to a third-party of, or sub-licensing of, Software or any third-party software. All Software, whether provided separately, installed on, or embedded in a Product, is licensed to Purchaser and not sold. Except as expressly stated in this Section, no right or license under any of Illumina's intellectual property rights is or are granted expressly, by implication, or by estoppel.

Purchaser is solely responsible for determining whether Purchaser has all intellectual property rights that are necessary for Purchaser's intended uses of this Product, including without limitation, any rights from third parties or rights to Application Specific IP. Illumina makes no guarantee or warranty that purchaser's specific intended uses will not infringe the intellectual property rights of a third party or Application Specific IP.

3 **Regulatory**. This Product has not been approved, cleared, or licensed by the United States Food and Drug Administration or any other regulatory entity whether foreign or domestic for any specific intended use, whether research, commercial, diagnostic, or otherwise. This Product is labeled For Research Use Only. Purchaser must ensure it has any regulatory approvals that are necessary for Purchaser's intended uses of this Product.

4 **Unauthorized Uses**. Purchaser agrees: (a) to use each Consumable only one time, and (b) to use only Illumina consumables/reagents with Illumina Hardware. The limitations in (a)-(b) do not apply if the Documentation or Specifications for this Product state otherwise. Purchaser agrees not to, nor authorize any third party to, engage in any of the following activities: (i) disassemble, reverse-engineer, reverse-compile, or reverse-assemble the Product, (ii) separate, extract, or isolate components of this Product or subject this Product or components thereof to any analysis not expressly authorized in this Product's Documentation, (iii) gain access to or attempt to determine the methods of operation of this

Product, or (iv) transfer to a third-party, or grant a sublicense, to any Software or any third-party software. Purchaser further agrees that the contents of and methods of operation of this Product are proprietary to Illumina and this Product contains or embodies trade secrets of Illumina. The conditions and restrictions found in these terms and conditions are bargained for conditions of sale and therefore control the sale of and use of this Product by Purchaser.

5 **Limited Liability. TO THE EXTENT PERMITTED BY LAW, IN NO EVENT SHALL ILLUMINA OR ITS SUPPLIERS BE LIABLE TO PURCHASER OR ANY THIRD PARTY FOR COSTS OF PROCUREMENT OF SUBSTITUTE PRODUCTS OR SERVICES, LOST PROFITS, DATA OR BUSINESS, OR FOR ANY INDIRECT, SPECIAL, INCIDENTAL, EXEMPLARY, CONSEQUENTIAL, OR PUNITIVE DAMAGES OF ANY KIND ARISING OUT OF OR IN CONNECTION WITH, WITHOUT LIMITATION, THE SALE OF THIS PRODUCT, ITS USE, ILLUMINA'S PERFORMANCE HEREUNDER OR ANY OF THESE TERMS AND CONDITIONS, HOWEVER ARISING OR CAUSED AND ON ANY THEORY OF LIABILITY (WHETHER IN CONTRACT, TORT (INCLUDING NEGLIGENCE), STRICT LIABILITY OR OTHERWISE).**

6 **ILLUMINA'S TOTAL AND CUMULATIVE LIABILITY TO PURCHASER OR ANY THIRD PARTY ARISING OUT OF OR IN CONNECTION WITH THESE TERMS AND CONDITIONS, INCLUDING WITHOUT LIMITATION, THIS PRODUCT (INCLUDING USE THEREOF) AND ILLUMINA'S PERFORMANCE HEREUNDER, WHETHER IN CONTRACT, TORT (INCLUDING NEGLIGENCE), STRICT LIABILITY OR OTHERWISE, SHALL IN NO EVENT EXCEED THE AMOUNT PAID TO ILLUMINA FOR THIS PRODUCT.**

7 **Limitations on Illumina Provided Warranties. TO THE EXTENT PERMITTED BY LAW AND SUBJECT TO THE EXPRESS PRODUCT WARRANTY MADE HEREIN ILLUMINA MAKES NO (AND EXPRESSLY DISCLAIMS ALL) WARRANTIES, EXPRESS, IMPLIED OR STATUTORY, WITH RESPECT TO THIS PRODUCT, INCLUDING WITHOUT LIMITATION, ANY IMPLIED WARRANTY OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, NONINFRINGEMENT, OR ARISING FROM COURSE OF PERFORMANCE, DEALING, USAGE OR TRADE. WITHOUT LIMITING THE GENERALITY OF THE FOREGOING, ILLUMINA MAKES NO CLAIM, REPRESENTATION, OR WARRANTY OF ANY KIND AS TO THE UTILITY OF THIS PRODUCT FOR PURCHASER'S INTENDED USES.**

8 **Product Warranty**. All warranties are personal to the Purchaser and may not be transferred or assigned to a third-party, including an affiliate of Purchaser. All warranties are facility specific and do not transfer if the Product is moved to another facility of Purchaser, unless Illumina conducts such move.

a **Warranty for Consumables**. Illumina warrants that Consumables, other than custom Consumables, will conform to their Specifications until the later of (i) 3 months from the date of shipment from Illumina, and (ii) any expiration date or the end of the shelf-life pre-printed on such Consumable by Illumina, but in no event later than 12 months from the date of shipment. With respect to custom Consumables (i.e., Consumables made to specifications or designs made by Purchaser or provided to Illumina by, or on behalf of, Purchaser), Illumina only warrants that the custom Consumables will be made and tested in accordance with Illumina's standard manufacturing and quality control processes. Illumina makes no warranty that custom Consumables will work as intended by Purchaser or for Purchaser's intended uses.

b **Warranty for Hardware**. Illumina warrants that Hardware, other than Upgraded Components, will conform to its Specifications for a period of 12 months after its shipment date from Illumina unless the Hardware includes Illumina provided installation in which case the warranty period begins on the date of installation or 30 days after the date it was delivered, whichever occurs first ("Base Hardware Warranty"). "Upgraded Components" means Illumina provided components, modifications, or enhancements to Hardware that was previously acquired by Purchaser. Illumina warrants that Upgraded Components will conform to their Specifications for a period of 90 days from the date the Upgraded Components are installed. Upgraded Components do not extend the warranty for the Hardware unless the upgrade was conducted by Illumina at Illumina's facilities in which case the upgraded Hardware shipped to Purchaser comes with a Base Hardware Warranty.

c **Exclusions from Warranty Coverage**. The foregoing warranties do not apply to the extent a non-conformance is due to (i) abuse, misuse, neglect, negligence, accident, improper storage, or use contrary to the Documentation or Specifications, (ii) improper handling, installation, maintenance, or repair (other than if performed by Illumina's personnel), (iii) unauthorized alterations, (iv) Force Majeure events, or (v) use with a third party's good not provided by Illumina (unless the Product's Documentation or Specifications expressly state such third party's good is for use with the Product).

d **Procedure for Warranty Coverage**. In order to be eligible for repair or replacement under this warranty Purchaser must (i) promptly contact Illumina's support department to report the non-conformance, (ii) cooperate with Illumina in confirming or diagnosing the non-conformance, and (iii) return this Product, transportation charges prepaid to Illumina following Illumina's instructions or, if agreed by Illumina and Purchaser, grant Illumina's authorized repair personnel access to this Product in order to confirm the non-conformance and make repairs.

e **Sole Remedy under Warranty**. Illumina will, at its option, repair or replace non-conforming Product that it confirms is covered by this warranty. Repaired or replaced Consumables come with a 30-day warranty. Hardware may be repaired or replaced with functionally equivalent, reconditioned, or new Hardware or components (if only a component of Hardware is non-conforming). If the Hardware is replaced in its entirety, the warranty period for the replacement is 90 days from the date of shipment or the remaining period on the original Hardware warranty, whichever is shorter. If only a component is being repaired or replaced, the warranty period for such component is 90 days from the date of shipment or the remaining period on the original Hardware warranty, whichever ends later. The preceding states Purchaser's sole remedy and Illumina's sole obligations under the warranty provided hereunder.

f **Third-Party Goods and Warranty**. Illumina has no warranty obligations with respect to any goods originating from a third party and supplied to Purchaser hereunder. Third-party goods are those that are labeled or branded with a third-party's name. The warranty for third-party goods, if any, is provided by the original manufacturer. Upon written request Illumina will attempt to pass through any such warranty to Purchaser.

9 **Indemnification**.

a **Infringement Indemnification by Illumina**. Subject to these terms and conditions, including without limitation, the Exclusions to Illumina's Indemnification Obligations (Section 9(b) below), the Conditions to Indemnification Obligations (Section 9(d) below), Illumina shall (i) defend, indemnify and hold harmless Purchaser against any third-party claim or action alleging that this Product when used for research use purposes, in accordance with these terms and conditions, and in accordance with this Product's Documentation and Specifications infringes the valid and enforceable intellectual property rights of a third party, and (ii) pay all settlements entered into, and all final judgments and costs (including reasonable attorneys' fees) awarded against Purchaser in connection with such infringement claim. If this Product or any part thereof, becomes, or in Illumina's opinion may become, the subject of an infringement claim, Illumina shall have the right, at its option, to (A) procure for Purchaser the right to continue using this Product, (B) modify or replace this Product with a substantially equivalent non-infringing substitute, or (C) require the return of this Product and terminate the rights, license, and any other permissions provided to Purchaser with respect this Product and refund to Purchaser the depreciated value (as shown in Purchaser's official records) of the returned Product at the time of such return; provided that, no refund will be given for used-up or expired Consumables. This Section states the entire liability of Illumina for any infringement of third party intellectual property rights.

b **Exclusions to Illumina Indemnification Obligations**. Illumina has no obligation to defend, indemnify or hold harmless Purchaser for any Illumina Infringement Claim to the extent such infringement arises from: (i) the use of this Product in any manner or for any purpose outside the scope of research use purposes, (ii) the use of this Product in any manner not in accordance with its Specifications, its Documentation, the rights expressly granted to Purchaser hereunder, or any breach by Purchaser of these terms and conditions, (iii) the use of this Product in combination with any other products, materials, or services not supplied by Illumina, (iv) the use of this Product to perform any assay or other process not supplied by Illumina, or (v) Illumina's compliance with specifications or instructions for this Product furnished by, or on behalf of, Purchaser (each of (i) – (v), is referred to as an "Excluded Claim").

c **Indemnification by Purchaser**. Purchaser shall defend, indemnify and hold harmless Illumina, its affiliates, their non-affiliate collaborators and development partners that contributed to the development of this Product, and their respective officers, directors, representatives and employees against any claims, liabilities, damages, fines, penalties, causes of action, and losses of any and every kind, including without limitation, personal injury or death claims, and infringement of a third party's intellectual property rights, resulting from, relating to, or arising out of (i) Purchaser's breach of any of these terms and conditions, (ii) Purchaser's use of this Product outside of the scope of research use purposes, (iii) any use of this Product not in accordance with this Product's Specifications or Documentation, or (iv) any Excluded Claim.

d **Conditions to Indemnification Obligations**. The parties' indemnification obligations are conditioned upon the party seeking indemnification (i) promptly notifying the other party in writing of such claim or action, (ii) giving the other party exclusive control and authority over the defense and settlement of such claim or action, (iii) not admitting infringement of any intellectual property right without prior written consent of the other party, (iv) not entering into any settlement or compromise of any such claim or action without the other party's prior written consent, and (v) providing reasonable assistance to the other party in the defense of the claim or action; provided that, the party reimburses the indemnified party for its reasonable out-of-pocket expenses incurred in providing such assistance.

e **Third-Party Goods and Indemnification**. Illumina has no indemnification obligations with respect to any goods originating from a third party and supplied to Purchaser. Third-party goods are those that are labeled or branded with a third-party's name. Purchaser's indemnification rights, if any, with respect to third party goods shall be pursuant to the original manufacturer's or licensor's indemnity. Upon written request Illumina will attempt to pass through such indemnity, if any, to Purchaser.

Revision History

Part #	Revision	Date	Description of Change
15023355	L	November 2013	Restructured content.
15023355	K	May 2013	Replaced the following reagents: • RMR for RMX
15023355	J	January 2013	Error correction; removed 1500 info from Performing a Sequencing Run sections.
15023355	H	November 2012	Revised to support HCS 2.0.
15023355	G	July 2012	Listed primer requirements for TruSeq HT libraries and Nextera libraries. Added list of supported dual-indexed libraries. Updated run setup for dual-indexed libraries to specify TruSeq Dual Index Sequencing Primer Box regardless of which sequencing primers are used for the run.
15023355	F	April 2012	Changed the run setup workflow: manifold gaskets must be replaced before performing the instrument maintenance wash instead of before loading the clustered flow cell. Added the following information: • Overviews of single-indexed and dual-indexed sequencing runs • Description of dual-indexed libraries • Example ICB volumes for dual-indexed runs • Reagent preparation instructions for SBS, paired-end, and indexing reagents, including sequencing primers required for dual-indexed libraries • Emphasis on using the correct version of TruSeq Dual Index Sequencing Primer Box based on flow cell type

Part #	Revision	Date	Description of Change
15023355	E	October 2011	Corrected the following information: • Approximate run duration for non-indexed runs • Number of required kits for dual-indexed runs, including maximum number of cycles performed with a 200-cycle kit • Instructions for splitting ICB for dual-indexed paired-end runs • Requirements of a valid sample sheet • Volumes of HP3 for indexed runs
15023355	D	October 2011	Updated software descriptions to HCS v1.5. Added instructions for setting up and performing dual-indexed runs for Nextera libraries.
15023355	C	June 2011	Added instructions to load 25 ml of laboratory-grade water to SBS position 2 for the automatic post-run rinse.
15023355	B	April 2011	Updated software descriptions to HCS v1.4. Added the following information: • Description of flow cell orientation check • Approximate run durations for HCS v1.4 • Version compatibility of software and reagent kit versions for various flow cell types • Information about reagents in the TruSeq SBS Kit v3, including description of SBS v3 alternative workflow for loading reagents • Instructions for idling the instrument Updated the following information: • Reagent positions with SBS v3 reagent names • Instructions for shutting down the instrument • Increased the amount of water required for the instrument maintenance wash
15023355	A	March 2011	Initial release.

Table of Contents

Revision History vii
Table of Contents ix

Chapter 1 Overview 1

Introduction 2
Delivery and Installation 4
HiSeq 1000 Configuration and Components 6
Single-Indexed Sequencing Overview 9
Dual-Indexed Sequencing Overview 11
Sample Sheet Overview 14
Available Disk Space 15
Sequencing Consumables 16
User-Supplied Consumables 17
Start the HiSeq 1000 18
HiSeq Software 19

Chapter 2 Perform a Sequencing Run 25

Introduction 26
Sequencing Workflow (8-Lane Flow Cell) 27
Prepare SBS Reagents 29
Prepare Indexing Reagents 30
Enter Run Parameters 31
Load and Prime Reagents 39
Pre-Run Setup Screens 40
Load a Flow Cell 50
Monitor the Run 56
Prepare Reagents for Read 2 58
Load Reagents for Read 2 59

Chapter 3 Post-Run Procedures 61

Introduction 62
Unload and Weigh Reagents 63
Performing an Instrument Maintenance Wash 64
Perform a Water Wash 68
Idle the Instrument 70
Shut Down the Instrument 71

Chapter 4 Real-Time Analysis 73

Real-Time Analysis Introduction 74
Monitor Runtime Statistics 75
Real-Time Analysis Output Data 77

Chapter 5 Troubleshooting 83

Introduction 84
Possible Run Setup Problems 85
Perform a Fluidics Check 86
Repeat Read 2 Primer Hybridization 87
If BaseSpace is Unavailable 88
Stop and Resume a Run 89
Pause a Run 93

Index 95

Technical Assistance 99

Overview

Chapter 1

Introduction 2
Delivery and Installation 4
HiSeq 1000 Configuration and Components 6
Single-Indexed Sequencing Overview 9
Dual-Indexed Sequencing Overview 11
Sample Sheet Overview 14
Available Disk Space 15
Sequencing Consumables 16
User-Supplied Consumables 17
Start the HiSeq 1000 18
HiSeq Software 19

Introduction

The Illumina HiSeq® System combines innovative engineering with proven SBS technology to set new standards in output, simplicity, and cost-effectiveness.

The HiSeq includes the following features:

- **Dual Surface Imaging**—The HiSeq uses a four camera epifluorescence system with cutting-edge scanning technology to enable dual surface imaging.
- **High-Capacity Reagent Chiller**—The reagent compartment is a high-capacity chiller that holds enough reagents for the entire sequencing run.
- **Integrated Fluidics for Paired-End Runs**—Integrated paired-end fluidics provide reagents from the reagent compartment to the flow cell for Read 2 resynthesis and for indexed sequencing.
- **Interface Control Options**—The instrument software interface provides options for setting up a run and operating the instrument using the touch screen monitor or the integrated keyboard.
- **Real-Time Analysis**—The instrument software performs real-time data analysis on the instrument computer, which allows you to monitor important quality metrics during the run and save valuable downstream analysis time. Downstream analysis of sequencing data can be performed with Illumina and third-party software applications, on IlluminaCompute computing and storage appliances, in the BaseSpace genomics cloud solution, or on a custom infrastructure.
- **BaseSpace Connectivity**—The HiSeq features an option to send instrument health and sequencing data to the BaseSpace genomics cloud solution in real time to streamline both instrument quality control and analysis. Real-time monitoring of runs from anywhere, including Illumina tech support, enables fast troubleshooting. BaseSpace facilitates collaboration by enabling you to share results instantly with anyone anywhere in the world. Free alignment and variant calling and BaseSpace app store provide many workflows that tailor analysis for diverse biological applications.

Audience and Purpose

This guide contains information about the HiSeq. It provides an overview of instrument components and software features along with instructions for performing a sequencing run.

This guide is for laboratory personnel and other individuals responsible for the following:

- Operating the instrument

- Performing sequencing runs
- Performing instrument and component maintenance
- Training personnel on the instrument

Delivery and Installation

An Illumina-authorized service provider delivers, uncrates, and places the HiSeq on the lab bench. The space and bench must be ready in advance of delivery. For more information, see Lab Bench Guidelines in the *HiSeq Site Prep Guide*, part # 15006407.

CAUTION
Allow only Illumina-authorized personnel to uncrate, install, or move the HiSeq. Mishandling of the instrument can affect the alignment or damage instrument components.

An Illumina representative installs and aligns the instrument. If you plan to connect the instrument to a data management system or remote network location, have the path for data storage selected before the date of installation. Having the path already selected allows your Illumina representative to test the data transfer process during installation. For more information, see Network Considerations in the *HiSeq Site Prep Guide*, part # 15006407.

CAUTION
After your Illumina representative has installed and aligned the HiSeq, ***do not*** relocate the instrument. Moving the instrument improperly can impact the optical alignment and compromise data integrity. If you need to relocate the HiSeq, contact your Illumina representative.

Crate Dimensions and Contents

The HiSeq 1000 and its components are shipped in three crates. Use the following dimensions to determine the minimum door width required to accommodate the shipping crates.

- Crate #1 contains the HiSeq 1000 instrument.
- Crate #2 contains one uninterruptible power supply (Japan, North America, or International version).
- Crate #3 contains the following items:
  - Workstation PC, monitor, and monitor arm
  - Waste bottle and reagent racks
  - Instrument panels
  - Power cords—One domestic power cord or three international power cords (UK, EU, and China)

Table 1 Crated Dimensions

Measurement	Crate #1: Instrument	Crate #2: UPS	Crate #3: Workstation, Covers, and Power Cords
Width	164 cm (64.5 in.)	77.5 cm (30.5 in.)	104 cm (41 in.)
Height	121 cm (47.5 in.)	51 cm (20 in.)	99.1 cm (39 in.)
Depth	104 cm (41 in.)	61 cm (24 in.)	81.3 cm (32 in.)
Weight	312 kg (688 lbs.)	80.3 kg (177 lbs.)	125 kg (275 lbs.)

HiSeq 1000 Configuration and Components

The HiSeq is shipped with a dedicated workstation that is customized to the latest system requirements. The computer workstation is a dedicated subsystem of the instrument and is not intended for use nor supported as a general-purpose workstation.

The HiSeq system comprises the HiSeq instrument. It includes a monitor, a dedicated workstation that is customized to the latest system requirements, a barcode scanner, a keyboard, a mouse, and a universal power supply.

Figure 1 HiSeq 1000 Main Compartments

- **A** Optics Module—Contains optical components that enable dual surface imaging of the flow cell, imaging A, C, G, and T at the same time using epifluorescence. The excitation laser beam passes through the objective and the fluorescence is collected through the same objective.
- **B** Flow Cell Compartment—Contains the vacuum-controlled flow cell stage, which holds the flow cell in place during sequencing runs.
- **C** Fluidics Compartment—Contains fluidics pumps that deliver reagents to the flow cell, and then to the waste container.
- **D** Status Bar—Uses three colors to indicate instrument status. Blue indicates that the instrument is running, orange indicates the instrument needs attention, and green indicates that the instrument is ready to begin the next run.
- **E** Reagent Compartment—Contains racks that hold reagents for sequencing runs, or wash solution for instrument washes.

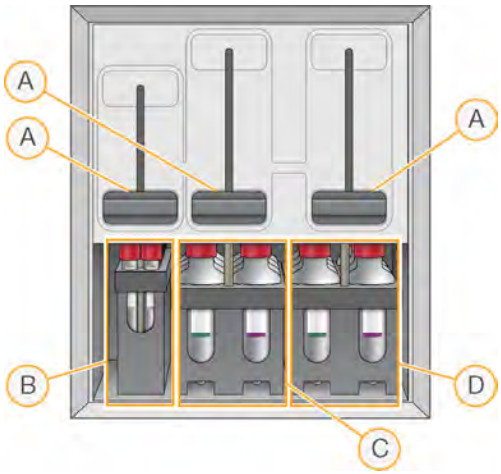
To set up a sequencing run on the HiSeq 1000, access the reagent compartment and the flow cell compartment.

Reagent Compartment

The reagent compartment is a high-capacity reagent chiller that holds three reagent racks: two for SBS reagents and one for paired-end reagents. Sipper handles lower the sippers into the reagent bottles.

- **SBS Reagent Racks**—SBS reagent racks hold 250 ml conical bottles. The reagent rack for flow cell A is located in the center position, and the rack for flow cell B is located in the far right position. Each reagent rack has numbered positions that correspond to connections on the fluidics pump.
- **Paired-end and Index Reagent Rack**—The reagent rack is located to the left of racks A and B. It has one row of numbered positions that holds 15 ml conical tubes containing paired-end reagents and indexing reagents.
- **Reagent Chiller**—The reagent chiller is designed to maintain reagent temperature at 4°C. The reagent chiller is not intended to cool down reagents. Reagents must be cooled before they are loaded onto the instrument.

Figure 2 Reagent Compartment

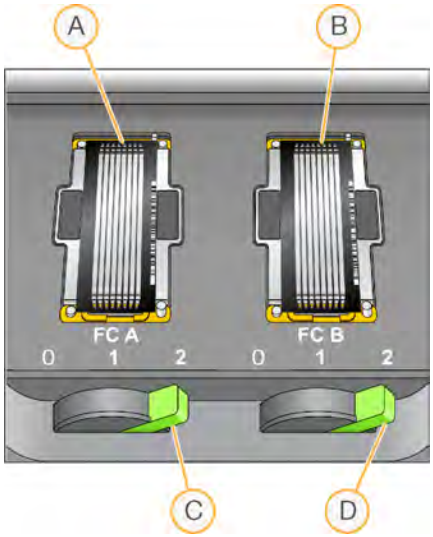


A Sipper Handles
B Reagent Rack for Paired-End, or Indexing Reagents
C Reagent Rack for SBS Reagents, Flow Cell A
D Reagent Rack for SBS Reagents, Flow Cell B

Flow Cell Compartment

The flow cell compartment houses the flow cell stage, the thermal stations, the vacuum system, and the fluidics connections to each flow cell.

Figure 3 Flow Cell Stage With High Output Flow Cells



A Flow Cell A
B Flow Cell B
C Flow Cell Lever A
D Flow Cell Lever B

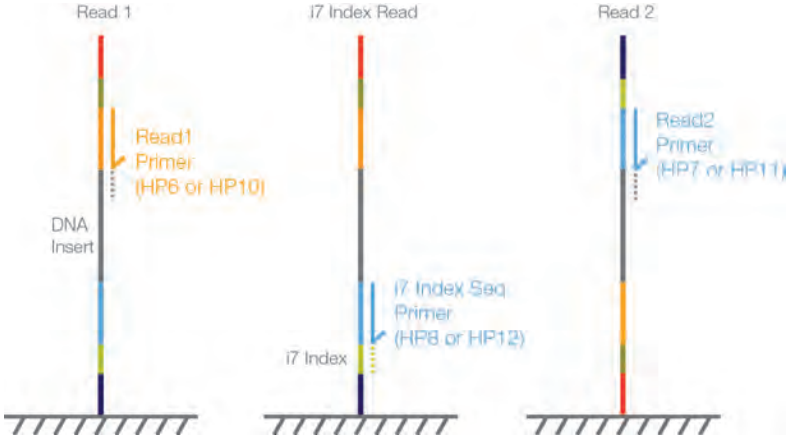
The flow cells are seated on the flow cell stage, which moves in and out of the optics module. For the dual flow cell system in the HiSeq 1000, flow cell A is on the left, and flow cell B is on the right. The flow cell is positioned on the flow cell holder with the inlet and outlet ports facing down, and held in place by a vacuum. The illuminated flow cell lever in front of each flow cell holder controls the vacuum. The flow cell lever turns green when the vacuum seal is secure.

You cannot open the flow cell compartment if the flow cell stage is not in forward-most position. The HiSeq Control Software controls the flow cell stage. The software positions the flow cell stage in the forward-most position when it prompts you to load or remove a flow cell.

Single-Indexed Sequencing Overview

Single-indexed sequencing includes one Index Read following Read 1.

Figure 4 Single-Indexed Sequencing



Read 1

Read 1 follows the standard Read 1 sequencing protocol using reagents provided in the TruSeq SBS Kit. The Read 1 sequencing primer is annealed to the template strand during the cluster generation process on the cBot.

- When sequencing dual-indexed Nextera libraries, use the sequencing primer provided in the TruSeq Dual Index Sequencing Primer Box, **HP10**. HP10 is used on the cBot in place of HP6. For more information, see the *cBot User Guide*, part # 15006165.
- When sequencing single-indexed TruSeq LT/v2 libraries or dual-indexed TruSeq HT libraries, you can use either HP6 or HP10.

Index Read Preparation

The Read 1 product is removed and the Index 1 (i7) sequencing primer is annealed to the same template strand, producing the Index 1 (i7) Read.

- When sequencing dual-indexed, use the appropriate sequencing primer provided in the TruSeq Dual Index Sequencing Primer Box, **HP12**, which you use in place of HP8.
- When sequencing single-indexed TruSeq LT/v2 libraries or dual-indexed TruSeq HT libraries, you can use either HP8 or HP12.

Index 1 (i7) Read

Following Index Read preparation, the Index 1 (i7) Read is performed.

- When sequencing single-indexed libraries, the Index Read consists of seven cycles of sequencing.
- When sequencing dual-indexed libraries (Nextera or TruSeq HT), the Index Read consists of eight cycles of sequencing.

Read 2 Resynthesis

The Index Read product is removed and the original template strand is used to regenerate the complementary strand. Then, the original template strand is removed to allow hybridization of the Read 2 sequencing primer.

- When sequencing dual-indexed Nextera libraries, use the sequencing primer provided in the TruSeq Dual Index Sequencing Primer Box, **HP11**, which you use in place of HP7.
- When sequencing single-indexed TruSeq LT/v2 libraries or dual-indexed TruSeq HT libraries, you can use either HP7 or HP11.

Read 2

Read 2 follows the standard paired-end sequencing protocol using reagents provided in the TruSeq SBS Kit.

Dual-Indexed Sequencing Overview

NOTE
Performing dual-indexed sequencing runs requires HiSeq Control Software (HCS) v1.5, or later.

Dual-indexed sequencing includes two Index Reads following Read 1.

Figure 5 Dual-Indexed Single-Read Sequencing

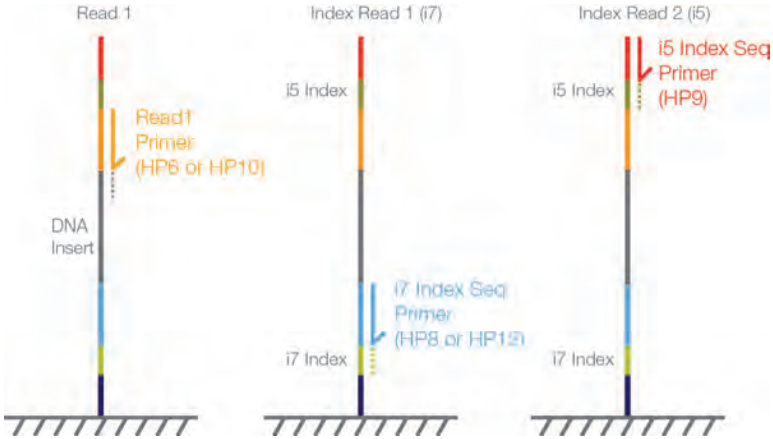
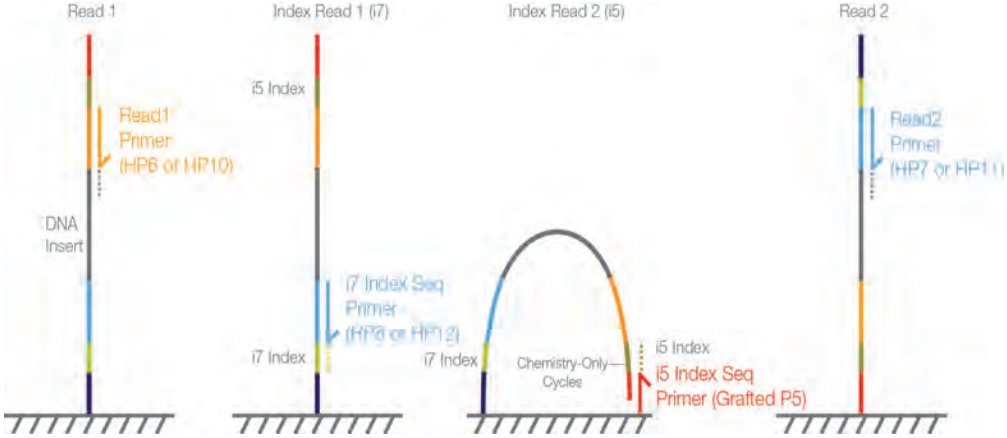


Figure 6 Dual-Indexed Paired-End Sequencing



Read 1

Read 1 follows the standard Read 1 sequencing protocol using reagents provided in the TruSeq SBS Kit. The Read 1 sequencing primer is annealed to the template strand during the cluster generation process on the cBot.

- When sequencing dual-indexed Nextera libraries, use the appropriate sequencing primer provided in the TruSeq Dual Index Sequencing Primer Box, **HP10**. This is used on the cBot in place of HP6. For more information, see the *cBot User Guide*, part # 15006165.
- When sequencing dual-indexed TruSeq HT libraries, you can use either HP6 or HP10.

Index Read Preparation

The Read 1 product is removed and the Index 1 (i7) sequencing primer is annealed to the same template strand, producing the Index 1 (i7) Read.

- When sequencing dual-indexed Nextera libraries, use the sequencing primer provided in the TruSeq Dual Index Sequencing Primer Box, **HP12**, which you use in place of HP8.
- When sequencing dual-indexed TruSeq HT libraries, you can use either HP8 or HP12.

Index 1 (i7) Read

Following Index Read preparation, the Index 1 (i7) Read is performed. When sequencing dual-indexed libraries (Nextera or TruSeq HT), the Index Read consists of eight cycles of sequencing.

Index 2 (i5) Read

The process for Index 2 (i5) Read is different for runs performed on single-read flow cells than for runs performed on paired-end flow cells:

- **Single-Read Flow Cells**—The Index 1 (i7) Read product is removed and the Index 2 (i5) Sequencing Primer Mix, **HP9**, is annealed to the same template strand. HP9 is provided in the TruSeq Dual Index Sequencing Primer Box and required for dual-indexed runs on single-read flow cells regardless of the library type (Nextera or TruSeq HT). The run proceeds through eight cycles of sequencing.
- **Paired-End Flow Cells**—The Index 1 (i7) Read product is removed. The Resynthesis Mix, **RMR**, deprotects the grafted P5 primer on the surface of the flow cell and the template anneals to the grafted P5 primer. The run proceeds through an additional

seven chemistry-only cycles (no imaging occurs), followed by eight cycles of sequencing.

Read 2 Resynthesis

The Index Read product is removed and the original template strand is used to regenerate the complementary strand. Then, the original template strand is removed to allow hybridization of the Read 2 sequencing primer.

- When sequencing dual-indexed Nextera libraries, use the sequencing primer provided in the TruSeq Dual Index Sequencing Primer Box, **HP11**, which you use in place of HP7.
- When sequencing dual-indexed TruSeq HT libraries, you can use HP7 or HP11.

Read 2

Read 2 follows the standard paired-end sequencing protocol using reagents provided in the TruSeq SBS Kit.

Dual-Indexed Libraries

Dual-indexed libraries are libraries prepared with adapters containing two indices:

- Index 1 (i7), an eight-base index adjacent to the P7 strand
- Index 2 (i5), an eight-base index adjacent to the P5 strand

During sample preparation, up to 12 unique Index 1 sequences are arrayed across the columns of a 96-well plate. And, up to eight unique Index 2 sequences are arrayed down the rows. This creates up to 96 uniquely dual-indexed adapters.

Sample Sheet Overview

The sample sheet is a user-generated file in *.csv format that stores information about a sequencing run. The sample sheet is copied into the run folder and later used for analysis.

Using a sample sheet is optional. If you choose to use a sample sheet, create one *before* you start the run.

NOTE

To view indexing results in Sequencing Analysis Viewer (SAV), you must load a sample sheet at the start of your run. SAV v1.8 requires a sample sheet to report indexing information. See the *Sequencing Analysis Viewer User Guide*, part # 15020619.

You can create your sample sheet in various software applications such as Excel or Notepad, or use the Illumina Experiment Manager.

Use the following guidelines when creating your sample sheet:

- All index sequences are specified in the Index field. If you are doing dual-indexed reads, you must separate all indices by a hyphen: ACCAGTAA–GGACATGA.
- If a sample does not contain an index sequence, leave the Index field blank.
- For a sample sheet to be valid, every cycle in an Index Read for every lane ***must*** have at least one base in the red channel (A,C) and one base in the green channel (G,T). This ensures proper image processing. Correct secondary analysis cannot be performed with an invalid sample sheet.

Available Disk Space

During the run at the end of imaging each lane, the software checks available disk space on the local D: and E: drives. The software does not check the network location during the run. If disk space drops below the safe threshold, the software pauses the run and places the flow cell in a safe state.

NOTE
Illumina recommends that you specify a network location for the output run folder. The output run folder is specified on the Storage screen. For more information, see *Storage Screen* on page 32.

If the disk space becomes low, make disk space available to continue the run. When sufficient disk space becomes available, the run automatically resumes.

The HiSeq 1000 instrument computer has a storage capacity of 2.7 TB per flow cell, enough space to hold a 209-cycle run of a high output flow cell.

Sequencing Consumables

Illumina-supplied consumables are required for sequencing on the HiSeq. The kits that you need depend on the type of run you perform and the type of libraries you are sequencing.

- For any type of run, you need at least one SBS kit.
- For indexed runs, you need one SR or PE cluster kit.
- For paired-end runs, indexed or non-indexed, you need one PE cluster kit.
- Some library types require sequencing primers that are provided in the TruSeq Dual Index Sequencing Primer Box. For more information, see the reagent preparation documentation associated with the cluster kit that you are using for your run.

User-Supplied Consumables

Consumables

Consumable	Supplier	Purpose
1 N NaOH	General lab supplier	Instrument maintenance wash.
Alcohol wipes, 70% Isopropyl or Ethanol, 70%	VWR, catalog # 15648-981 General lab supplier	Cleaning the flow cell and flow cell stage.
Centrifuge tubes, 250 ml	Corning, catalog # 430776	Instrument maintenance wash and water wash.
Conical tubes, 15 ml	Corning, catalog # 430052	Collecting and measuring waste volumes.
Disposable gloves, powder-free	General lab supplier	General use.
Lab tissue, low-lint	VWR, catalog # 21905-026	Cleaning the flow cell holder.
Lens paper, 4 x 6 in.	VWR, catalog # 52846-001	Cleaning the flow cell.
Water, laboratory-grade, 18 M Ohm	Millipore	SBS reagent rack, position 2. Water wash.

Start the HiSeq 1000

Follow best practices when you turn on, turn off, and operate the computer:

- Do not power up the computer while the instrument is on.
- Allow the instrument to be off for a full minute before turning it back on.
- Never turn off the instrument while the instrument software is running.
- Connect the instrument USB cable, monitor USB, and keyboard USB to the back of the server; connect the barcode scanner and mouse to the front of the server.
- Do not use port manager to delete ports.

Use the following instructions to start the HiSeq 1000 and instrument computer.

1 Start the instrument control computer.

2 Log on to the operating system using the default user name and password. Wait until it has loaded.
   - User name: sbsuser
   - Password: sbs123

   If the default values do not work, consult your IT personnel for the user name and password for your site.

3 Switch the main power switch to the ON position. If you are facing the front of the instrument, the power switch is on the left side.

4 Wait for the instrument drive called "DoNotEject" to initialize. A window opens when the drive is initialized. Close the window.

NOTE
Never eject the "DoNotEject" flash drive located inside the instrument chassis, or modify the files on it. This drive contains hardware configuration files and initializes whenever the instrument is turned on.

5 To ensure adequate disk space, archive the data on the instrument computer from previous runs to a network location.

6 Open HCS using the shortcut icon on the computer desktop. HCS takes a few minutes to initialize. When the software is initialized, the Welcome screen opens and the initialization icon

appears on the bottom left of the screen.

HiSeq Software

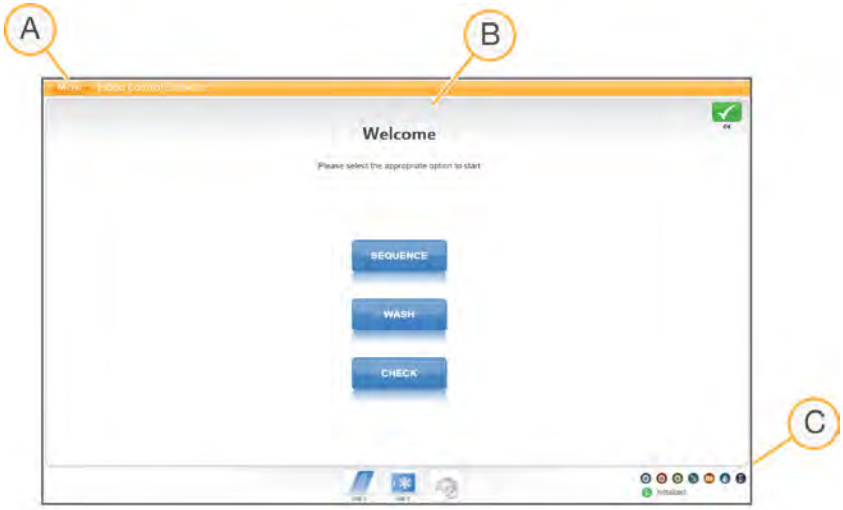
Three software applications are installed on the instrument computer:

- **HiSeq Control Software**—The HiSeq Control Software (HCS) interface guides you through the steps to specify run parameters, and load the flow cell and reagents before starting the run. During the run, HCS provides a summary of quality statistics throughout the run.
- **Real-Time Analysis**—The integrated primary analysis software, Real-Time Analysis (RTA), performs base calling and assigns a quality score to each base for each cycle. For more information, see *Real-Time Analysis Introduction* on page 74.
- **Sequencing Analysis Viewer**—The Sequencing Analysis Viewer (SAV) provides detailed quality statistics and updated metrics as the run progresses. For more information, see *Sequencing Analysis Viewer* on page 75.

HiSeq Control Software (HCS) Interface

The HCS interface opens to run configuration screen.

Figure 7 HCS Interface, Welcome Screen



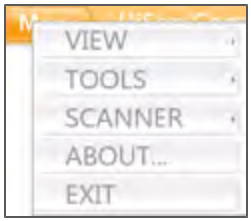
A Welcome Screen Menu Button
B Main Interface Panel
C Activity Indicators

Welcome Screen Menu

The Welcome screen menu button, located in the upper-left corner of the Welcome screen, provides the following options:

- **View**—Provides options to view the interface in full screen or in a window, or to minimize the interface.
- **Tools**—Provides access to the Options window and Show Log file:
  - **Options**—From the Options window, you can define the run Id template, default folder locations, and the LIMS server, user name, and password. See *Menu Options Window* on page 20.
  - **Show Log File**—The Show Log file is a text file used for troubleshooting.
- **Scanner**—Activates the command to initialize the software manually.
- **About**—Provides information about instrument hardware, software versions, and technical support contact information.
- **Exit**—Closes the HCS interface.

Figure 8 Menu Button



Menu Options Window

From the Options window, you can define the run ID template, default folder locations, and the LIMS server, user name, and password.

Figure 9 Menu Options Window

Run ID Template—The name used to name a run folder

Default Output Folder—The default location for flow cell A runs to be saved. This can be changed on a per run basis

Default Output Folder2—The default location for flow cell B runs to be saved. This can be changed on a per run basis

Default Temp Folder1—The location to which temporary files can be written during a run

Run Setup Folder—Where the HiSeq picks up LIMS sample forms

LIMS Server—The server name for interactions with supported Illumina LIMS

LIMS Username—The LIMS user name HiSeq uses when authenticating to Illumina LIMS

LIMS Password—The LIMS password HiSeq uses when authenticating to Illumina LIMS

Send instrument health information to Illumina to aid technical support—Gives permission to the instrument to send information to BaseSpace for each run. All information remains confidential. Illumina recommends that you keep this feature enabled and never shares your information. Run sample data is sent only if you specifically indicate on the Storage screen that you want to send run sample data. See *Storage Screen* on page 32 for more information.

Start Screen Options

The options on the Welcome screen are as follows:

- Sequence
- Wash
- Check

Sequence—Select **Sequence** to begin the steps to set up a sequencing run. From the Sequence button, you can start a new run or resume an existing run.

Figure 10 Sequence Command Options

- **New Run**—The software guides you through setting run parameters, loading and priming reagents, loading the flow cell, performing fluidics checks, and starting the run.
- **Resume Run**—The software guides you through selecting the existing run folder and setting parameters for resuming the run.

Wash—Select **Wash** to initiate an instrument wash. From the Wash button, you can start a Water Wash or a Maintenance Wash.

Figure 11 Wash Command Options

- **Water Wash**—The water wash flushes water through the system. A water wash is recommended before you start a new run if the instrument has been idle for one day or more. For more information, see *Perform a Water Wash* on page 68.
- **Maintenance Wash**—The maintenance wash flushes water, then NaOH, and then water through the system. This wash is recommended after each sequencing run. *Performing an Instrument Maintenance Wash* on page 64

Check—Select **Check** to open the fluidics check screen and confirm proper flow during instrument installation or fluidics troubleshooting.

Activity Indicators

The Welcome screen contains a series of icons in the lower-right corner of the screen that indicate which activity the instrument is performing.

Figure 12 Activity Indicators. From left to right, they represent the X, Y, and Z motors, electronics functionality, the camera, the fluidics system, and processing functions.

Figure 13 Activity Indicators. From left to right, they represent flow cell A temperature, reagent chiller temperature, BaseSpace cloud status, and flow cell B temperature.

Status Icons

Status icons are located in the upper-right corner of each screen, and notify you of any change in conditions during run setup or during the run.

Status Icon	Status Name	Description
	Status OK	No change. System is normal.
	Information	Information only. No action is required
	Attention	Information that might require attention.
	Warning	Warnings do not stop a run, but might require action before proceeding.
	Error	Errors usually stop a run and generally require action before proceeding with the run.

When a change in condition occurs, the icon changes and blinks to alert you. If this happens, select the icon to open the status window, which contains a general description of the condition. Select **Acknowledge** to accept the message and **Close** to close the dialog box.

You can filter the types of messages that appear in the status window by selecting the icons along the top margin of the window. Selecting an icon toggles the condition to show or to hide.

NOTE

For more information about known issues related to errors and warnings, see the release notes for the version of instrument control software you are using.

Perform a Sequencing Run

Chapter 2

Introduction 26
Sequencing Workflow (8-Lane Flow Cell) 27
Prepare SBS Reagents 29
Prepare Indexing Reagents 30
Enter Run Parameters 31
Load and Prime Reagents 39
Pre-Run Setup Screens 40
Load a Flow Cell 50
Monitor the Run 56
Prepare Reagents for Read 2 58
Load Reagents for Read 2 59

Introduction

To perform a sequencing run on the HiSeq 1000, you first prepare reagents for your run and then set up the run using the HCS interface. Run setup steps include: entering run parameters, loading and priming reagents, loading the high output flow cell, and performing a fluidics check.

- Reagents for Read 1 and the Index Reads are loaded during run setup before starting the run.
- Reagents for Read 2 resynthesis are loaded after the completion of Read 1 and the Index Reads.
- Illumina recommends that you prepare and load fresh ICB for Read 2.

Types of Runs

You can perform six types of runs on the HiSeq 1000:

Run Type	Read 1 Cycles	Index 1 (i7) Read Cycles	Index 2 (i5) Read Cycles	Read 2 Cycles	Total Cycles
Single-Read, Non-Indexed	≤ 101	--	--	--	≤ 101
Single-Read, Single-Indexed	≤ 101	7 * 8 **	--	--	≤ 108 * ≤ 109 **
Single-Read, Dual-Indexed	≤ 101	8	8	--	≤ 117
Paired-End, Non-Indexed	≤ 101	--	--	≤ 101	≤ 202
Paired-End, Single-Indexed	≤ 101	7 * 8 **	--	≤ 101	≤ 209 * ≤ 210 **
Paired-End, Dual-Indexed	≤ 101	8	7 + 8 ***	≤ 101	≤ 225

* Number of cycles for single-indexed libraries
** Number of cycles for dual-indexed libraries
*** Index 2 Read of a paired-end dual-indexed run includes seven additional chemistry-only cycles

For more information, see *Single-Indexed Sequencing Overview* on page 9 and *Dual-Indexed Sequencing Overview* on page 11.

Sequencing Workflow (8-Lane Flow Cell)

Prepare SBS reagents. This includes thawing and inverting all reagents. For details about SBS reagents, see the SBS reagent prep guides for the reagents that you use for your experiment. To find the guides, go to the Illumina website: support.illumina.com/sequencing/kits.ilmn.

For indexed runs:
Prepare indexing reagents. Make sure that you prepare the appropriate sequencing primers for your library and type of run. For details about indexing reagents, see the indexing reagent prep guides for the reagents that you are using for your experiment. To find the guides, go to the Illumina website: support.illumina.com/sequencing/kits.ilmn.

Using HCS, enter parameters for your run.

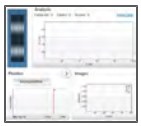
When prompted, load SBS reagents for Read 1 and Read 2, except ICB (recommended).

For indexed runs:
Load indexing reagents. For dual-indexed runs, load reagents for both Index Reads.

With a used flow cell on the instrument, confirm proper flow as prompted by the HCS user interface.

Prime SBS reagents and measure priming waste.

Load a flow cell that was previously clustered on the cBot. Confirm proper flow as prompted by the HCS user interface.



Start your sequencing run. After cycle 1, inspect the First Base Report (optional setting), and then continue Read 1.

For indexed runs:

Following Read 1, the run proceeds to the Index Read (single-indexed runs) or two Index Reads (dual-indexed runs).

For paired-end runs:

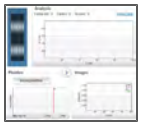
Prepare paired-end reagents and Read 2 reagents. For details about reagents, see the reagent prep guides for the kits that you are using for your run. To find the guides, go to the Illumina website: support.illumina.com/sequencing/kits.ilmn.

Prepare fresh ICB for Read 2 (recommended).

For paired-end runs:

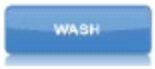
Load paired-end reagents onto the paired-end rack.

Load fresh ICB in position 1 (recommended).



For paired-end runs:

Continue the run. The software automatically primes paired-end reagents and performs Read 2 resynthesis and Read 2.



When the run is complete, unload and weigh reagents.

Perform an instrument maintenance wash.

Visit the HiSeq specifications page on the Illumina website for information about run duration and other performance specifications at www.illumina.com/systems/hiseq_2000_1000/performance_specifications.ilmn.

Prepare SBS Reagents

The first step in setting up a run is to prepare SBS reagents. Reagent preparation requires overnight thawing at 2° to 8°C, or about 90 minutes in a room temperature deionized water bath. When thawed, reagents are ready for use. Incorporation Mix requires preparation before use.

For more information, see the reagent preparation instructions associated with the reagents that you are using for your run. Go to support.illumina.com/sequencing/kits.ilmn to find any documentation that you may need.

NOTE

TruSeq SBS v3 reagents enable an alternative workflow of loading all SBS reagents at the start of a paired-end sequencing run for both Read 1 and Read 2. Although this alternative SBS workflow provides acceptable sequencing performance in Read 2, Illumina recommends that you prepare and load fresh ICB at the beginning of Read 2 for optimal performance.

Prepare Indexing Reagents

Indexed sequencing requires indexing reagents based on the type of libraries you are sequencing and the number of Index Reads that you perform.

Indexing reagents take less than 30 minutes to prepare.

For more information, see the reagent preparation instructions associated with the reagents that you are using for your run. Go to support.illumina.com/sequencing/kits.ilmn to find any documentation that you may need.

Enter Run Parameters

When you select **Sequence** | **New Run** from the Welcome screen, the software opens the run setup screens that guide you through the run setup steps, starting with the screens on the Run Configuration tab.

Each run configuration screen contains drop-down lists, checkboxes, or text fields. Use the hand-held barcode scanner to scan the flow cell or reagent kit ID, or enter the ID using the touch screen keyboard. The keyboard icon is located to the right of the text fields.

When you have finished entering the required information on each screen, the Next button becomes active. Select **Next** to move to the next screen or select **Back** to return to the previous screen. At any time during the run setup steps, you can select **Cancel** to abort the run setup and return to the Welcome screen.

Prepare SBS reagents and indexing reagents before setting up the run. For instructions, see the reagent prep documentation for the kits that you are using.

- From the HCS Welcome screen, select **Sequence** | **New Run**. The Scan screen opens.

Gasket Change Screen

At the end of a run, the software opens the Gasket Change screens that guides you through a maintenance wash.

1. **Load Gasket** — You are prompted to load a new gasket.
2. **Load Wash Reagents** — You are prompted to load wash reagents.
3. **Load Wash Flow Cell** — You are prompted to load a non-sequencing flow cell and enter a wash flow cell ID.
4. **Fluidics Check** — You are prompted to select a solution. Use default values for Volume, Aspiration Rate, and Dispense Rate.
5. **Wash** — The instrument performs a wash.
6. **Load Solvent** — You are prompted to load NaOH in all positions for washing.
7. **Solvent Wash** — The instrument performs a solvent wash.
8. **Load Wash Reagents** — You are prompted to load wash reagents.
9. **Wash** — The instrument performs a wash.

Maintenance Wash Screen

If necessary, HCS prompts you to run a maintenance wash before a run. In all cases, a maintenance wash is recommended after every run.

1. **Load Wash Reagents** — You are prompted to load wash reagents.
2. **Load Wash Flow Cell** — You are prompted to load a non-sequencing flow cell and enter a wash flow cell ID.
3. **Fluidics Check** — You are prompted to select a solution. Use default values for Volume, Aspiration Rate, and Dispense Rate.
4. **Wash** — The instrument performs a wash.
5. **Load Solvent** — You are prompted to load NaOH in all positions for washing.
6. **Solvent Wash** — The instrument performs a solvent wash.
7. **Load Wash Reagents** — You are prompted to load wash reagents.
8. **Wash** —The instrument performs a wash.

Storage Screen

The Storage screen enables you to define where the system outputs and stores your run data.

1. Select **Use BaseSpace for storage and analysis** if you want to save your data to a BaseSpace account. If you select this option, The system prompts you to enter a My Illumina account email and password. Zip BCL files is selected by default. Illumina recommends that you also save files locally. To save files locally, select **Save to an out put folder** and enter a path. The **Run Monitoring Only** option sends only system health information to BaseSpace.

NOTE

For every run, your HiSeq 1000 attempts to send at least general run metrics to BaseSpace. Metrics are sent anonymously, associated with the HiSeq 1000 from which they came, not to any particular user. Metrics include interoperation files (that SAV uses) and global files such as RunInfo.xml and RunParameters.xml. No data files are sent unless you specify for the system to do so.

Permissions for this feature are enabled by default, and Illumina recommends that you leave them enabled. To disable permissions, select **Menu** | **Tools** | **Options**, and then deselect **Send system health information to Illumina to aid technical support**. You can also view terms in the Options dialog box.

If permissions for this feature are not enabled, HCS prompts you to accept Terms and Conditions. If you do not accept the terms and conditions, you cannot use BaseSpace. If you are not using BaseSpace, go back to the previous screen and make sure that the BaseSpace option is de-select (otherwise the Next button is not enabled).

2 In **Save to an output folder**, browse to and select the path of your run folder.

3 Select **Zip BCL files** to reduce the footprint of disc space needed for the run. If you are using BaseSpace, this option is selected by default.

NOTE

If you are using BaseSpace or an updated version of CASAVA for data analysis, zip the BCL files.

4 Select **Bin QScores** to reduce the footprint of disc space needed for the run.
This is done by grouping Q-scores together over a wider range of values. For technical details regarding how data quality score resolution can be reduced without sacrificing score accuracy or standard analysis and variant calling performance, find the white paper on www.Illumina.com titled, *Reducing Whole-Genome Data Storage Footprint*.

5 Select a setting from the **Save Auxiliary Files** drop-down list:
- Select **Save All Thumbnails** to save tile level and full thumbnails. Thumbnail images are a combination of eight images from each swath, combined in one thumbnail image. A swath is a column of tiles, and there are three swaths per lane per surface.
- Select **Save Tile Thumbnails** to save tile level thumbnails only. Tile thumbnails show just the tile rather than the whole swath.

6 Select **Next**.

Flow Cell Setup Screen

The Flow Cell Setup screen is the run configuration screen with which you record information about your run, starting with the flow cell ID.

NOTE
It is critical that you scan the flow cell barcode or accurately enter the flow cell ID when you set up your run. The software uses the flow cell ID to determine flow cell type and reagent compatibility.

1. Scan the flow cell barcode or enter the flow cell ID (barcode number) of the flow cell to be sequenced.
2. Confirm the flow cell type, which is automatically selected based on the flow cell ID. The software uses this information to confirm flow cell compatibility with instrument software and run components.
3. Enter the experiment name. This name appears at the top of each interface screen to help identify the sequencing run in progress.
4. Enter a user name.
5. Select the lane containing the control, if applicable. Otherwise, select **None**.
6. Select **Next**.

Advanced Screen

The Advanced screen includes settings for first base confirmation and indicating alignment lanes.

1. Select the **Confirm First Base** checkbox to generate a First Base Report confirmation. Selecting this option requires that you confirm the results before the run can continue. If you do not select the checkbox, the First Base Report is generated as usual and the run continues without showing the First Base Report confirmation dialog box. You can access the First Base Report from the run folder at any time during the run.
2. Select the control lane on your flow cell containing the control library from the drop-down list. Otherwise, select **None**.
3. Select or deselect **Align to PhiX** for each lane of the flow cell.
   This enables real-time reporting of error rates for PhiX control lanes or PhiX spike-in lanes. By default, all lanes are selected for alignment by Real-Time Analysis (RTA).

Clear the checkbox for any lanes that do not have PhiX added to the sample so that RTA does not align and calculate error rates for that lane, resulting in arbitrarily high error rates. For more information, see *Using a PhiX Control for HiSeq Sequencing Runs*, which is available on the HiSeq support page on the Illumina website at www.illumina.com.

4 Select **Keep Intensity Files** for later reanalysis or custom processing (optional).
Select the checkbox to save cluster intensity files (*.cif) for later reanalysis, troubleshooting, or custom processing. Saving intensity files is not required for on-instrument analysis. Enabling this option significantly increases the size of the data output folder. By default, this option is not selected.

5 Select **Next**.

Recipe Screen

From the information that you enter on the Recipe screen, the software creates a recipe specific to the run. The recipe is automatically copied to the run folder and saved in the Recipe subfolder. The saved recipe includes only the number of cycles for each read and index; it does not save the options for the control lane, PhiX alignment, images, or cifs.

1 Select an indexing option:
- **No Index**—Performs a non-indexed single-read or paired-end run.
- **Single Index**—Performs a single-read or paired-end run with one Index Read (Index 1 (i7) Read).
- **Dual Index**—Performs a single-read or paired-end run with two Index Reads (Index 1 (i7) Read and Index 2 (i5) Read).
- **Custom**—Performs a single-read or paired-end run with a custom number of cycles for the Index Reads.
  If you select Dual Index or Custom, select a **Flow Cell Format**: **Single Read** or **Paired End**.
  A dual-indexed single-read run can be performed on either a single-read flow cell or a paired-end flow cell with recipe adjustments. Your selection in HCS, paired-end or single-read flow cell, determines the recipe. Always use the appropriate dual-index sequencing primers box according to your flow cell type.

  NOTE
  A dual-indexed paired-end run must be performed on a paired-end flow cell.

2 Enter the number of cycles you plan to sequence for Read 1 and Read 2, if applicable.

For the **Custom** indexing option, enter the number of cycles you plan to sequence for the Index Reads.

For paired-end runs, enter the **same** number of cycles for Read 2 as you entered for Read 1. Or, if you selected the **Custom** indexing option, enter the number of cycles for each Index Read. (Read lengths do not need to be identical.)

NOTE
It is possible to perform a dual-indexed single-read run using a paired-end flow cell containing paired-end libraries. To do so, set up a ***dual-indexed paired-end run*** and enter 0 (zero) for the number of cycles in Read 2. This stops the run after the Index 2 (i5) Read.

3 Confirm the chemistry selections for your run:

a **SBS**—This setting is determined by the flow cell ID.

NOTE
The software defaults to the compatible kit versions based on the flow cell ID. For example, the ID for HiSeq Flow Cell v3 defaults to the TruSeq SBS Kit v3.

b **Index**—This setting is determined by the indexing option selected. Specify the kit name to make sure that the correct number of indexing cycles are applied to your run.

- **For non-indexed or single-indexed libraries**—Select **Multiplexing Primer Box**. This sets up your run to perform a seven-cycle Index Read.
- **For dual-indexed libraries**—Select **TruSeq Dual Index Sequencing Primer Box**. This sets up your run to perform an eight-cycle Index Read.

NOTE
Select TruSeq Dual Index Sequencing Primer Box setting

4 Select the **Use Existing Recipe** checkbox only if you want to use a custom recipe. Otherwise, allow the software to create your recipe based on information you provide on the run setup screens.

Illumina recommends that you use the recipe wizard to build your recipe based on the information you enter on the Recipe screen.

Sample Sheet Screen

In the **Sample Sheet** field, enter the path of a valid sample sheet for your run. When the run begins, the software copies the sample sheet to the root level of the run folder. Unless you are using BaseSpace, a sample sheet is optional. For indexed runs, the sample sheet is required if you want indices to be reported in SAV; if you are running BaseSpace, a sample

sheet is required. For more information about sample sheets, see *Sample Sheet Overview* on page 14.

NOTE
To view indexing results in Sequencing Analysis Viewer (SAV), you must load a sample sheet at the start of your run. SAV v1.8 requires a sample sheet to properly report indexing information. For more information, see the *Sequencing Analysis Viewer User Guide*, part # 15020619.

1 Enter the path or browse to a valid sample sheet for your run. For indexed runs, the sample sheet is required if you want indices to be reported in SAV; a sample sheet is also required if you are using BaseSpace.

2 Select **Next**.

Reagents Screen

The Reagents screen records information about the reagents used for your run.

1 Scan or enter the Reagent Kit ID. You can enter the kit ID from either box 1 or box 2.

2 For indexed runs, scan or enter the Indexing Reagent Kit ID.

3 For paired-end runs, scan or enter the Reagent Kit ID.

4 Select the SBS reagent kit you are using for your run:
- Select **200 Cycles** for a 200 cycle kit
- Select **50 Cycles** for a 50 cycle kit.
- Select Used Kit for a partial kit, and then enter the number of SBS cycles remaining, or the number of cycles that reagents are expected to last.

NOTE
If you are combining four 50-cycle SBS kits for a dual-indexed run, select **Used Kit** instead of 50 Cycles to set the cycle counter correctly.

5 The Cycles Remaining field is populated automatically depending on the SBS kit you are using. If you are using a partial kit, enter the number of cycles reagents are expected to last. The software counts down the number of cycles entered. When the cycles are low, the software prompts you to load fresh reagents.

NOTE
If you are starting a new run using less than a full reagent kit, make sure that you are present when new reagents are needed.

- Select **Prime SBS Reagents** to prime reagents before you start a run. Always prime reagents before loading a new flow cell.

6 Select **Next**.

Review Screen

Review your run information. If it is correct, select **Next**.

Select **Back** if you need to change any entries.

Load and Prime Reagents

The next step is to load sequencing reagents. The software interface guides you through the process.

Pre-Run Setup Screens

After you have entered the required information in the run configuration screens, the pre-run setup screens open. Pre-run setup screens include: Load Reagents, Load Prime Flow Cell, Prime Fluidics Check, and Prime.

Load SBS Reagents

The Load Reagents screen prompts you to load SBS reagents. If you specified a number of cycles for indexing, the software prompts you to load indexing reagents on the paired-end rack.

Make sure that SBS reagents are thoroughly thawed and ready to load onto the instrument.

After Read 1 of a paired-end run, the software pauses so that you can load paired-end reagents. Paired-end reagents must be loaded before Read 2 resynthesis.

NOTE
To prepare for the post-run rinse at the end of a sequencing run, it is important that you load 25 ml of PW1 or laboratory-grade water in position 2 when you load SBS reagents at the beginning of your run.

The post-run rinse does ***not*** replace the post-run instrument wash.

Illumina-Supplied Consumables

- Eight funnel caps (Provided in the TruSeq Cluster Kit)

User-Supplied Consumables

- One 250 ml bottle (Corning, catalog # 430776)

Procedure

1 Record the weight of each reagent on the lab tracking form.

NOTE
Weighing reagents before and after a sequencing run confirms proper reagent delivery.

2 Open the reagent compartment door.

3 Raise the sippers for the sequencing reagent rack using the following motion:
   a Pull the sipper handle towards you.
   b Push the sipper handle up while pulling it towards you.

c Release the sipper handle into the slot on the top end of the groove. Make sure that the sipper handle rests securely in the slot.

4 Slide the reagent rack out of the reagent compartment.

5 Place each reagent bottle onto the rack in the associated numbered position. Make sure that the conical end of the bottle rests in the indentation on the base of the rack.

Table 2 SBS Reagent Positions

Position	Reagent	Description
1	ICB	Incorporation Mix
2	PW1 (25 ml)	Wash Buffer
3	SRE	Scan Mix Reagent
4	SBS Buffer 1 (SB1)	High Salt Buffer
5	SBS Buffer 2 (SB2)	Incorporation Wash Buffer
6	SBS Buffer 2 (SB2)	Incorporation Wash Buffer
7	CMR	Cleavage Mix Reagent
8	SBS Buffer 3 (SB3)	Cleavage Buffer

6 Add 25 ml of PW1 or laboratory-grade water to the bottle in position 2.

NOTE
HCS v2.0 and later includes an automatic post-run rinse following the completion of the sequencing run. To prepare for the post-run rinse, it is important that you load 25 ml of PW1 or laboratory-grade water in position 2 when you load SBS reagents at the beginning of your run.

The post-run rinse does ***not*** replace the post-run instrument wash.

7 Remove the cap from each reagent bottle and replace it with a funnel cap.

CAUTION
After handling the bottle of CMR, discard your gloves and replace them with a new pair.

8 Slide the reagent rack into the reagent compartment, aligning the rack with the raised guide on the floor of the compartment.

9 Lower the sippers into the sequencing reagent bottles as follows:

a Pull the sipper handle towards you.

b Lower the sipper handle while pulling it towards you.

c Visually inspect the sippers to make sure that they do not bend as they lower into the funnel caps.

d Release the sipper handle into the slot on the bottom end of the groove. Make sure that the sipper handle rests securely in the slot.

10 Select the checkbox labeled **PW1 (25 mL) loaded in Position 2**.

11 Do one of the following:
- If you are performing an indexed run, select **Next**. The software prompts you to load indexing reagents.
- If you are performing a non-indexed run, close the reagent compartment door and select **Next**. The software proceeds to priming reagents.

Load Indexing Reagents

If you are performing an indexed run, load indexing reagents before starting the run.

1 Before beginning, record the weight of each reagent on the lab tracking form.

2 Raise the sippers for the paired-end reagent rack using the following motion:
a Pull the handle towards you.
b Push the handle up while pulling it towards you.
c Release the handle into the slot on the top end of the groove. Make sure that the handle rests securely in the slot.

3 Slide the reagent rack out of the reagent compartment using the rack handle.

4 Place each reagent tube onto the rack in the associated numbered position for the type of run that you are performing.

5 Place tubes filled with deionized water into rack positions that are not used.

6 Remove the caps from each reagent tube.

7 Slide the reagent rack into the reagent compartment, aligning the rack with the raised guide on the floor of the compartment.

8 Lower the sippers into the tubes on the paired-end reagent rack as follows:
a Pull the handle towards you.
b Lower the handle while pulling it towards you.
c Visually inspect the sippers to make sure that they do not bend as they lower into the tubes.
d Release the handle into the slot on the bottom end of the groove. Make sure that the handle rests securely in the slot.

9 Close the reagent compartment door.

10 Select **Next**.

Prime Reagents

Perform the following steps to prime reagents:

- *Clean the Flow Cell Holder* on page 43
- *Load the Used Flow Cell* on page 45
- *Confirm Proper Flow* on page 47
- *Position Tubing and Prime Reagents* on page 48

User-Supplied Consumables

- Lens cleaning tissue
- 70% ethanol or alcohol wipes
- Low-lint lab tissue
- Used flow cell

Clean the Flow Cell Holder

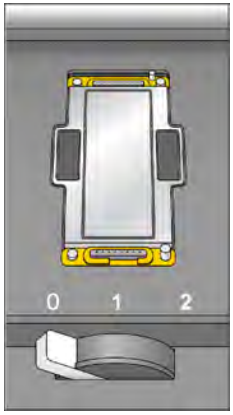
1 Open the flow cell compartment door.

CAUTION

Do not place fluids on the flow cell compartment door or on the flow cell stage when the door is open. Spills in this area can damage the instrument.

2 Make sure that the flow cell lever is in the OFF position.

Figure 14 Flow Cell Lever in Position 0



3 Put on a new pair of powder-free latex gloves.

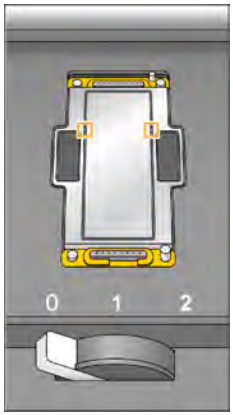
4 If the flow cell from a previous run is present, remove it and set aside. It can be used to confirm proper flow before loading the clustered flow cell.

5 Using an alcohol wipe or a lint-free tissue moistened with ethanol or isopropanol, carefully wipe the surface of the flow cell holder until it is clean.

CAUTION
Do not allow alcohol to drip into the vacuum holes or around the manifolds. Use a low-lint lab tissue to dry the stage, if necessary.

6 Visually inspect the flow cell holder to make sure that it is free of lint and the vacuum holes are free of obstructions.

Figure 15 Vacuum Hole Locations



Load the Used Flow Cell

The Load Prime Flow Cell screen prompts you to load a *used* flow cell for the priming step. After loading a used flow cell, confirm that the vacuum is engaged.

NOTE
Illumina recommends that you use the flow cell from a previous run for priming reagents on a subsequent run or for a post-run maintenance wash.

1 Rinse the used flow cell with laboratory-grade water. Dry it with lens cleaning tissue or lint-free tissue.

2 Clean the flow cell using alcohol wipes and lens cleaning tissue.

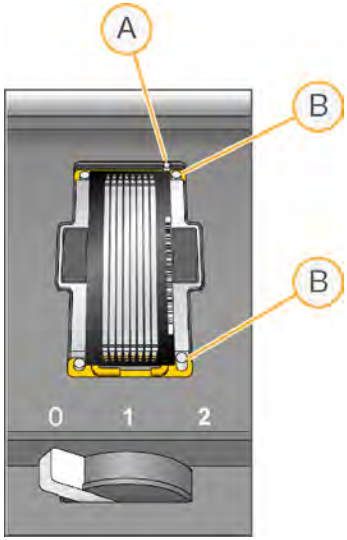
NOTE
Do not remove or replace the manifold gaskets at this time.

3 Place the used flow cell on the flow cell holder with the inlet and outlet ports facing *down* and the barcode on the right. Make sure that the arrow on the left edge of the flow cell, which indicates flow direction, points towards the instrument.

4 Gently slide the flow cell towards the top and right guide pins until it stops.

NOTE
Remove your hand from the flow cell before engaging the vacuum switch. This prevents any possible alignment drift over time.

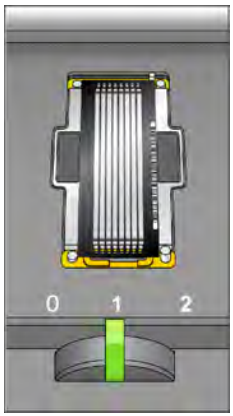
Figure 16 Flow Cell Positioned Against Top and Right Guide Pins.



A Top Guide Pin
B Right Guide Pins

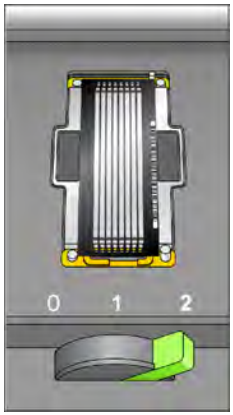
5 Slowly move the flow cell lever to position 1. This engages the vacuum and secures the flow cell into position. When the flow cell lever is green, the vacuum is engaged.

Figure 17 Flow Cell Lever in Position 1



6 Wait for about 5 seconds, and then slowly move the flow cell lever to position 2 (far-right). When the flow cell lever is solid green, the manifolds are in position and the flow cell is ready for use.

Figure 18 Flow Cell Lever in Position 2



7 Make sure that the **Vacuum Engaged** checkbox is selected on the load prime flow cell screen, and then select **Next**.

Confirm Proper Flow

After the used flow cell is loaded, check for proper flow. Checking for proper flow confirms that the flow cell and gaskets are properly installed and the manifold is engaged.

1 Select solution 2 (laboratory-grade water) from the drop-down list.

CAUTION
You can use water to confirm proper flow on a used flow cell only. Never use water to confirm proper flow on a clustered flow cell.

2 Confirm the following default values:
- Volume: **250**
- Aspirate Rate: **250**
- Dispense Rate: **2000**

3 Select **Pump**.

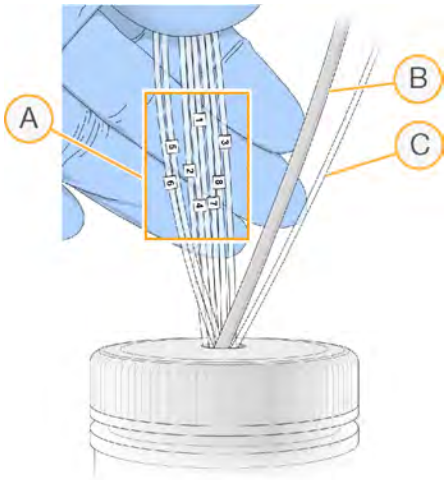
4 Visually inspect the flow cell for bubbles passing through the lanes and leaks near the manifolds.

If you see excessive bubbles, check the gaskets for obstructions, reduce the aspirate rate to 100, and pump another 250 µl of water to the flow cell. If problems persist, remove the flow cell, repeat the cleaning steps, and reload the flow cell.

Position Tubing and Prime Reagents

To position tubing, loosen and remove the eight lines of waste tubing for the appropriate flow cell from the waste container..

Figure 19 Waste Lines for a HiSeq 1000



A Flow Cell Waste Lines for Reagent Positions 1–8
B Condensation Pump Tubing (Do not remove)
C Paired-End Priming Pump Tubing (Do not remove)

1 Place each waste tubing into an empty 15 ml tube, one line per tube. Priming waste is collected and measured after the priming step.

2 Select **Next**. The priming screen opens and the priming step starts automatically. You can monitor the progress of the priming step from the priming screen.

Prime Fluidics Check and Prime Reagents

The Prime Fluidics Check screen prompts you to confirm proper flow before priming reagents. Select **Pump** to start the fluidics check.

You can pause or cancel the fluidics check from this screen:

- Select **Pause** to pause the fluidics check temporarily. The Pause button changes to the Resume button.
- Select **Resume** to continue.
- Select **Stop** to cancel the fluidics check.

After the fluidics check, the Prime screen opens.

From the Prime screen, you can monitor the priming step in progress. Select **Start Prime** to begin, or select **Skip Prime** if you have already primed reagents.

You can pause or cancel the priming step from this screen:

- Select **Pause** to temporarily pause the priming step. The Pause button changes to the Resume button.
- Select **Resume** to continue.
- Select **Stop** to stop priming.
- Select **Cancel** to abort the run setup and return to the Welcome screen.

When the priming step is complete, the initiate run screens open automatically.

1. When the priming step is complete, measure the collected priming waste and confirm that the volume is 2 ml from each reagent position primed for a total of **14 ml**. Record the results on the lab tracking form.

NOTE
The color of sequencing reagent waste might be brown in appearance. You also might see a slight discoloration on the funnel caps or in any spills. The change in waste color is not toxic and does not affect run performance.

2. Return the waste tubing to the waste container before proceeding.
3. Select **Next**. You are ready to load the clustered flow cell.

Load a Flow Cell

The next step is to remove the used flow cell and load the flow cell that you want to sequence.

Perform the following steps to load the flow cell:

- *Remove the Used Flow Cell* on page 50
- *Clean the Flow Cell Holder* on page 51
- *Clean the Flow Cell* on page 52
- *Load the Flow Cell* on page 53
- *Confirm Proper Flow* on page 55

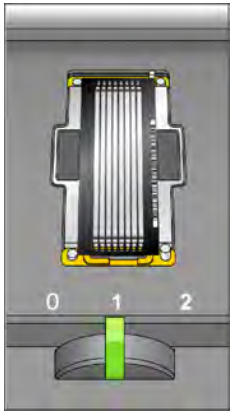
User-Supplied Consumables

- Lens cleaning tissue
- 70% ethanol or alcohol wipes
- Low-lint lab tissue

Remove the Used Flow Cell

1 Open the flow cell compartment door.

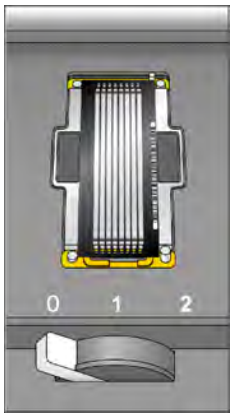
Figure 20 Flow Cell Lever in Position 1



2 Slowly move the flow cell lever to position 1 to disengage the manifolds.

3 Slowly move the flow cell lever to position 0 to disengage the vacuum seal and release the flow cell.

Figure 21 Flow Cell Lever in Position 0



4 Lift the used flow cell from the flow cell holder.

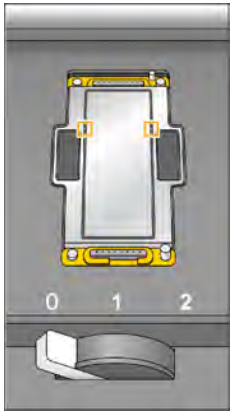
Clean the Flow Cell Holder

1 Put on a new pair of powder-free latex gloves.

2 Using an alcohol wipe or a lint-free tissue moistened with ethanol or isopropanol, carefully wipe the surface of the flow cell holder until it is clean.

CAUTION
Do not allow alcohol to drip into the vacuum holes or around the manifolds. Use a low-lint lab tissue to dry the stage, if necessary.

Figure 22 Inspect Vacuum Holes



3 Visually inspect the flow cell holder to make sure that it is free of lint and the vacuum holes are free of obstructions.

Clean the Flow Cell

1 Rinse the flow cell with laboratory-grade water and dry it with a lens cleaning tissue.

2 Fold an alcohol wipe to approximately the size of the flow cell.

3 Hold the edges of the clustered flow cell with two fingers. Make sure that the inlet and outlet ports are facing *up*.

4 Wipe off each side of the flow cell with a single sweeping motion. Repeat, refolding the alcohol wipe with each pass, until the flow cell is clean.

5 Dry the flow cell using a dry lens cleaning tissue.

CAUTION
If you clean the flow cell while it is lying on the benchtop, you could easily apply too much pressure and break the flow cell. Illumina recommends cleaning the flow cell while holding the edges between your fingers.

6 Protect the flow cell from dust until you are ready to load it onto the instrument.

Load the Flow Cell

NOTE
Do not replace the manifold gaskets at this time. Leave the gaskets that were installed before the instrument maintenance wash in place when loading the flow cell. You will replace manifold gaskets after the current sequencing run is completed, before the instrument maintenance wash.

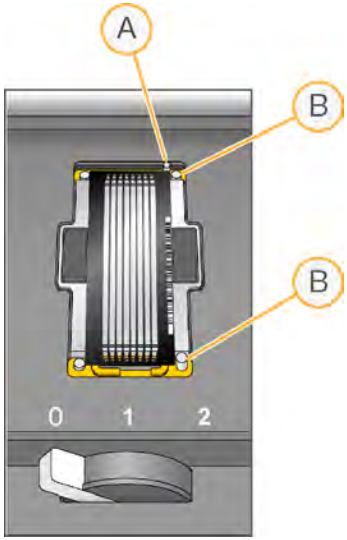
Do not perform more than one sequencing run on the same set of manifold gaskets.

1 Place the flow cell on the flow cell holder with the inlet and outlet ports facing *down* and the barcode on the right. Make sure that the arrow on the left edge of the flow cell, which indicates flow direction, points towards the instrument.

NOTE
The flow cell has a mechanically keyed corner, which provides a visual orientation for loading the flow cell. Install the flow cell so that the keyed corner is on the output end of the flow cell facing towards the instrument, and on the left side of the flow cell by lane 1.

2 Gently slide the flow cell towards the top and right guide pins until it stops.

Figure 23 Flow Cell Positioned Against Top and Right Guide Pins

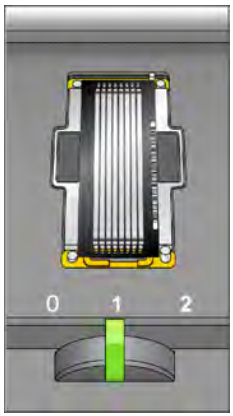


A Top Guide Pin
B Right Guide Pins

NOTE
Remove your hand from the flow cell before engaging the vacuum switch. This prevents possible alignment drift over time.

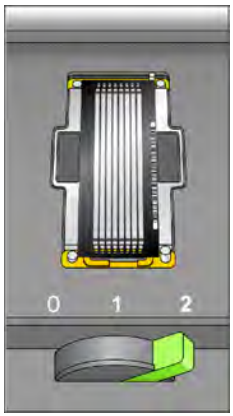
3 Slowly move the flow cell lever to position 1. This engages the vacuum and secures the flow cell into position. When the flow cell lever is green, the vacuum is engaged.

Figure 24 Flow Cell Lever in Position 1



4 Wait for about 5 seconds, and then slowly move the flow cell lever to position 2. When the flow cell lever is solid green, the manifolds are in position and the flow cell is ready for use.

Figure 25 Flow Cell Lever in Position 2



5 Make sure that the **Vacuum Engaged** checkbox is selected on the load sequencing flow cell screen.

Confirm Proper Flow

After loading the flow cell, check for proper flow using the fluidics check screen. Checking for proper flow confirms that the flow cell and gaskets are properly installed and the manifold is engaged.

1 Select solution 5 (SB2) from the drop-down list.

2 Enter the following default values:
- Volume: **250**
- Aspirate Rate: **250**
- Dispense Rate: **2000**

3 Select **Pump**.

4 Visually inspect the flow cell for bubbles passing through the lanes and leaks near the manifolds.

If you see excessive bubbles, check the manifold gaskets for obstructions, and repeat the process.

a Select solution 6 (SB2) to avoid depleting SB2 from position 5.

b Reduce the aspirate rate to 100, and pump another 250 µl of SB2 to the flow cell.

5 After you have confirmed proper flow, select **Next** to proceed. Make sure that the flow cell lever is green, and then close the flow cell compartment door.

6 Confirm that the **Vacuum Engaged** and **Door Closed** checkboxes are selected, and then select **Next**.

7 Select **Start** to start the sequencing run.

Close Door Screen

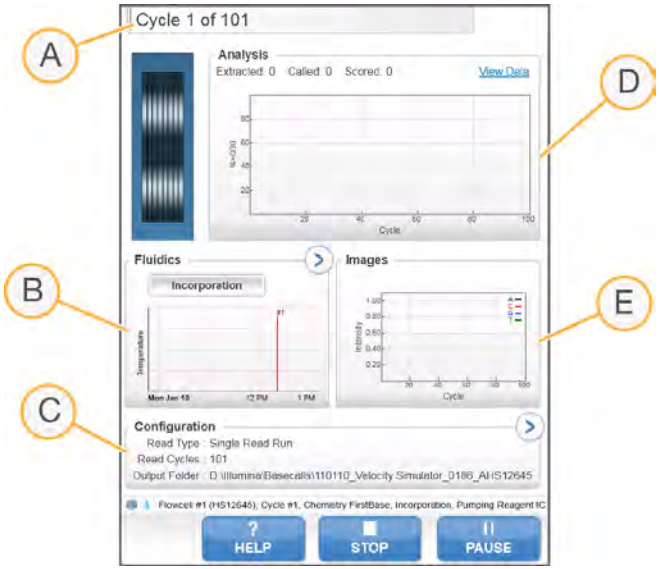
The Close Door screen prompts you to close the flow cell compartment door. The system confirms that the vacuum is engaged and that the flow cell compartment door is closed. The checkboxes are selected automatically when the system is ready.

Monitor the Run

Use the run overview screen to monitor on-instrument analysis, fluidics, and imaging.

- **Progress Bar**—Use the progress bar to monitor how many cycles have been completed.
- **Flow Cell Image**—Use the flow cell image to monitor which lanes have been imaged for that cycle.
- **Analysis Graph**—Use the analysis graph to monitor quality scores by cycle.
- **Images Graph**—Use the images graph to monitor intensities by cycle.
- **Fluidics Graph**—Use the arrow button to expand the fluidics section and monitor chemistry steps in the protocol.

Figure 26 Run Monitoring Screen Overview



A Run Progress
B Fluidics and Temperature
C Run Configuration
D Analysis
E Images

NOTE
You might notice an intermittent clicking sound coming from the right side of the instrument during operation. This is normal. The clicking sound indicates that the refrigeration condensation pump is working.

First Base Report

The First Base Report confirmation dialog box opens automatically after cycle one is complete, if you selected **Confirm First Base** on the scan screen during run setup. The run pauses at this step.

If you did not select the checkbox, the run continues after first base incorporation without prompting you to review the First Base Report.

1 Review the First Base Report from the First Base Report confirmation dialog box or by opening First_Base_Report.htm from the root level of the run folder.

2 If the results are satisfactory, select **Continue** to continue your run. If results are not satisfactory, abort the run.

Sequencing Analysis Viewer

You can monitor your run using the Sequencing Analysis Viewer, which launches automatically after image analysis begins. For more information, see *Sequencing Analysis Viewer* on page 75.

For a detailed description of available metrics and installation instructions, see the *Sequencing Analysis Viewer User Guide*, part # 15020619.

Prepare Reagents for Read 2

Before the completion of Read 1 and any Index Reads, prepare reagents for Read 2 resynthesis and fresh ICB for Read 2. Reagents take less than 30 minutes to prepare.

For more information, see the reagent preparation documentation associated with the kits that you are using for your run. Go to support.illumina.com/sequencing/kits.ilmn to find any documentation that you may need.

Load Reagents for Read 2

Prepare paired-end reagents and ICB for Read 2 before setting up the run. For instructions, see the reagent prep documentation for the kits that you are using.

After completion of Read 1 and any Index Reads, the software prompts you to load paired-end reagents for Read 2 resynthesis and freshly prepared ICB for Read 2.

Load Paired-End Reagents

1 Record the weight of each reagent on the lab tracking form.

2 Raise the sippers for the paired-end reagent rack using the following motion:
   a Pull the handle towards you.
   b Push the handle up while pulling it towards you.
   c Release the handle into the slot on the top end of the groove. Make sure that the handle rests securely in the slot.

3 Slide the reagent rack out of the reagent compartment using the rack handle.

4 Place each of the reagent tubes onto the rack in the associated numbered positions depending on the type of run you are performing.

Table 3 Paired-End Reagent Positions

Position	Reagent	Description
11	LMX2	Linearization Mix 2
12	BMX	Blocking Mix
13	AMX2	Amplification Mix 2
14	APM2	AMX2 Premix
15	AT2	100% Formamide
16	HP7 or HP11	Read 2 Sequencing Primer
18	HP3	Denaturation Solution
19	HT2	Wash Buffer

NOTE
You do not need to load RMR for dual-indexed paired-end runs. RMR is used during the Index 2 (i5) Read and is not used again during Read 2 resynthesis.

5 Remove the caps from each reagent tube.

6 Slide the reagent rack into the reagent compartment, aligning the rack with the raised guide on the floor of the compartment.

7 Lower the sippers into the paired-end reagent tubes as follows:
   a Pull the handle towards you.
   b Lower the handle while pulling it towards you.
   c Visually inspect the sippers to make sure that they do not bend as they lower into the tubes.
   d Release the handle into the slot on the bottom end of the groove. Make sure that the handle rests securely in the slot.

Load ICB for Read 2

1 Record the weight of the reagent on the lab tracking form.

2 Raise the sippers for the sequencing reagent rack using the following motion:
   a Pull the sipper handle towards you.
   b Push the sipper handle up while pulling it towards you.
   c Release the sipper handle into the slot on top end of the groove. Make sure that the sipper handle rests securely in the slot.

3 Slide the reagent rack out of the reagent compartment.

4 Remove the existing ICB reagent bottle from position 1 of the reagent rack and remove the funnel cap from the bottle.

5 Place the funnel cap on the new bottle of ICB and load the bottle in position 1, making sure that the conical end of the bottle rests in the indentation on the base of the rack.

6 Slide the reagent rack into the reagent compartment, aligning the rack with the raised guide on the floor of the compartment.

7 Lower the sippers into the sequencing reagent bottles as follows:
   a Pull the sipper handle towards you.
   b Lower the sipper handle while pulling it towards you.
   c Visually inspect the sippers to make sure that they do not bend as they lower into the funnel caps.
   d Release the sipper handle into the slot on the bottom end of the groove. Make sure that the sipper handle rests securely in the slot.

8 Close the reagent compartment door, and then select **Next** to resume the run.

Post-Run Procedures

Chapter 3

Introduction 62
Unload and Weigh Reagents 63
Performing an Instrument Maintenance Wash 64
Perform a Water Wash 68
Idle the Instrument 70
Shut Down the Instrument 71

Introduction

Post-run procedures include the removal and weighing of reagents, and an instrument wash.

Regular instrument washes ensure continued performance in the following ways:

- Flushes any remaining reagents and sample from the fluidics lines and sippers
- Prevents salt accumulation and crystallization in the fluidics lines and sippers
- Prevents cross-contamination from the previous run.

NOTE

A water wash is performed at the end of each run to wash the system and check fluidics. HCS does not allow you to start a new run unless a water wash or required maintenance wash has been performed within the last 24 hours. Every 10 days, HCS prompts you to perform a maintenance wash without regard to when you last performed a maintenance wash.

Unload and Weigh Reagents

1. Open the reagent compartment door.
2. Raise the sippers for the reagent rack and clustering rack you are loading using the following motion:
   a. Pull the sipper handle towards you.
   b. Push the sipper handle up while pulling it towards you.
   c. Release the sipper handle into the slot on the top end of the groove. Make sure that the sipper handle rests securely in the slot.
3. Slide the reagent rack out of the reagent compartment using the rack handle.
4. Remove each bottle from the reagent rack and record the weight on the lab tracking form.

NOTE

The color of sequencing reagent waste might be brown in appearance. You also might see a slight discoloration on the funnel caps or in any spills. The change in waste color is not toxic and does not affect run performance.

WARNING

A component in the clustering kit contains formamide, an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact.

Dispose of containers and any unused contents in accordance with the governmental safety standards for your region.

For more information, see the MSDS for this kit, at www.illumina.com/msds.

Performing an Instrument Maintenance Wash

To ensure optimal instrument performance, perform an instrument maintenance wash every 10 days, after each sequencing run, and prior to switching between Rapid Run mode and High Output mode. Change the gasket at the time of the maintenance wash. Regardless of which mode you are running, you will always have a 10 port gasket in the front manifold and an 8 port gasket in the back manifold. The maintenance wash consists of three wash steps:

- First water wash—Flushes the system with laboratory-grade water.
- NaOH wash—Washes the system with NaOH.
- Final water wash—Flushes the system with laboratory-grade water to remove NaOH.

Positions	Delivered Volume
Eight SBS positions	20 minutes
Eight SBS positions and ten clusters	60 minutes

User-Supplied Consumables

- Ethanol wipes
- 24 bottles, 250 ml (Corning, catalog # 430776), eight bottles per wash step
- 30 tubes, 15 ml (Corning, catalog # 430052), 10 tubes per wash step
- Laboratory-grade water
- 1 N NaOH
- 3 eppendorfs per flow cell for sample loading station wash steps

First Water Wash

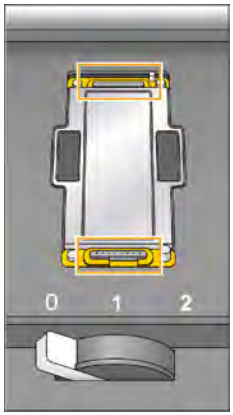
1. From the HCS Welcome screen select **Wash** | **Maintenance**.
2. Select **Yes** to wash PE reagent positions. Otherwise, select **No**. Select **Next** to proceed.
3. Load the instrument with laboratory-grade water as follows:
   a. Fill eight 250 ml bottles with more laboratory-grade water than there were reagents present at the beginning of the run to make sure that the sippers are rinsed.
   b. If you are washing clusters, load ten 15 ml tubes with 10 ml laboratory-grade water.
   c. Load the bottles and tubes onto the instrument in the appropriate reagent rack.

4 Remove the flow cell from the flow cell stage, and set it aside. Reload this flow cell before starting the instrument wash.

NOTE
Do not change gaskets during the NaOH and second wash steps. Gaskets are to remain installed until the sequencing run is complete.
Always install new manifold gaskets before performing an instrument maintenance wash.

5 Make sure that you have protective gloves on, and remove the front and back gaskets. Do so by applying light pressure with your finger to one side of the gasket. The other side pops up. Use tweezers to grasp and remove each gasket.

Figure 27 Remove Used Manifold Gaskets



6 Position two new gaskets in the slots on the front end and back end of the flow cell holder.

7 Load the flow cell that you removed to install the new gaskets. Select **Next**.

8 Perform a fluidics check:
   a Select solution 2 from the drop-down list.
   b Enter the following default values:
      — Volume: **250**
      — Aspirate Rate: **250**
      — Dispense Rate: **2000**
   c Select **Pump**.
   d Visually inspect the flow cell for bubbles passing through the lanes and leaks near the manifolds.

9 Loosen and remove the eight lines of waste tubing from the waste container. Make sure that you do not include the lines from the condensation pump or the cluster priming pump.

10 Bundle the eight lines of waste tubing with parafilm, making sure to keep all of the ends even. Place the bundled tube ends into a 250 ml bottle.

11 Select **Next**.

12 When the wash is complete, measure the delivered volume.
The following tables list the wash volumes from the eight lines of waste tubing. They do not include the priming pump wash volume.

Positions	Delivered Volume
Eight SBS positions	32 ml
Eight SBS positions and ten clusters	72 ml

NaOH Wash

CAUTION
For safety purposes, wear protective eyewear when working with large volumes of NaOH.

1 Load the instrument with 1 N NaOH as follows:
   a Load eight 250 ml bottles with 5 ml of NaOH.
   b If you are washing cluster positions, load ten 15 ml tubes with 5 ml NaOH.

2 Load the bottles and tubes onto the instrument in the appropriate reagent rack.

3 Select **Next** to start the NaOH wash.

4 When the NaOH wash is complete, measure the delivered volume.

Positions	Delivered Volume
Eight SBS positions	16 ml
Eight SBS positions and 10 cluster positions	36 ml

Final Water Wash

1 Load the instrument with laboratory-grade water as follows:

a Load eight 250 ml bottles with at least 20 ml of laboratory-grade water.

b If you are washing paired-end positions, load ten 15 ml tubes with at least 10 ml laboratory-grade water.

CAUTION

Do not reuse the same water or wash bottles that you used for the first wash step. The water from the first wash step might be contaminated with reagents that were present on the sippers.

2 Load the bottles and tubes onto the instrument in the appropriate reagent rack.

3 Select **Next** to start the final water wash.

4 When the final water wash is complete, measure the delivered volume.

Positions	Delivered Volume
Eight SBS positions	32 ml
Eight SBS positions and 10 cluster positions	72 ml

5 Unwrap the waste lines and return them to the waste container.

Perform a Water Wash

Perform a water wash if the instrument has been idle for one day or more before you begin a new sequencing run.

NOTE
HCS does not allow you to start a new run if the instrument has been idle for more than 24 hours.

Before the start of every run, be sure to carry out the recommended fluidics check as you are prompted by the software, to ensure that fluidics are working properly.

Illumina-Supplied Consumables

- Laboratory-grade water

User-Supplied Consumables

- Eight 250 ml bottles (Corning, catalog # 430776)
- Ten 15 ml conical tubes (Corning, catalog # 430052)
- One Eppendorf tube flip-top tube for each flow cell

Procedure

1. From the HCS Welcome screen select **Wash** | **Water**.
2. Load the instrument with laboratory-grade water as follows:
   a. Fill eight 250 ml bottles with 20 ml laboratory-grade water.
   b. If you are washing paired-end positions, load ten 15 ml tubes with 10 ml laboratory-grade water.

   NOTE
   Manifold gaskets do not require replacement before a water wash.
3. Make sure that a used flow cell is loaded. Load a used flow cell, if necessary. Select **Next**.

NOTE
Illumina recommends a fluidics check if you loaded a used flow cell for the wash.

4. If you loaded a used flow cell in the previous step, perform a fluidics check:
   a. Select solution 2 from the drop-down list.
   b. Enter the following default values:

- Volume: **250**
- Aspirate Rate: **250**
- Dispense Rate: **2000**

c Select **Pump**.

d Visually inspect the flow cell for bubbles passing through the lanes and leaks near the manifolds.

5 Loosen and remove the eight lines of waste tubing for the appropriate flow cell from the waste container. Make sure that you do not include the eight lines for the opposite flow cell, or the lines for the condensation pump or the cluster priming pumps.

6 Bundle all of the lines of waste tubing with parafilm, making sure to keep all of the ends even. Place the bundled tube ends into a 250 ml bottle.

7 Select **Next** to start the water wash.

Positions	Approximate Run Time
Eight SBS positions	20 minutes
Eight SBS positions and 10 paired-end positions	60 minutes

Positions	Approximate Run Time
Eight SBS positions	26 minutes
Eight SBS positions and 10 paired-end positions	52 minutes

8 When the wash is complete, measure the delivered volume. Record the delivery volume on the lab tracking form.

Positions	Delivered Volume
Eight SBS positions	32 ml
Eight SBS positions and 10 cluster positions	72 ml

9 Return the waste tubing to the waste bottle.

Idle the Instrument

If the instrument is not going to be used within the next 10 days, prepare it to sit idle. If it is not going to be used for more than 10 days, see *Shut Down the Instrument* on page 71.

1. Perform a complete maintenance wash to flush the system fully. For more information, see *Performing an Instrument Maintenance Wash* on page 64.
2. Leave the flow cell on the flow cell stage with the flow cell lever in position 2. This leaves the manifolds in the raised position.
3. Load 10 ml of laboratory-grade water in each reagent position in the reagent racks. Then lower the sippers into the water.
4. Leave the instrument on.
5. When you are ready to use the instrument for another run, perform a water wash. For more information, see *Perform a Water Wash* on page 68.

Shut Down the Instrument

Turn off the instrument only if it is not going to be used for more than 10 days. If the instrument is going to be used within the next 10 days, see *Idle the Instrument* on page 70. If you turn off the instrument, you do not need to turn off the PC.

Use the following procedure to prepare fluidics safely and shut down the system.

1. Perform a complete maintenance wash to fully flush the system. For more information, see *Performing an Instrument Maintenance Wash* on page 64.
2. Remove the flow cell(s) from the flow cell stage.
3. Using an alcohol wipe or a lint-free tissue moistened with ethanol or isopropanol, carefully wipe the surface of the flow cell holder until it is clean.

CAUTION

Do not allow alcohol to drip into the vacuum holes or around the manifolds. Use a low-lint lab tissue to dry the stage, if necessary.

4. Load 10 ml of laboratory-grade water in each reagent position in the reagent racks. Then lower the sippers into the water.
5. Turn off the instrument.
6. When you are ready to restart the instrument, load water in all reagent positions, turn on the instrument, and perform a water wash. For more information, see *Perform a Water Wash* on page 68.

Real-Time Analysis

Chapter 4

Real-Time Analysis Introduction 74
Monitor Runtime Statistics 75
Real-Time Analysis Output Data 77

Real-Time Analysis Introduction

The integrated primary analysis software, Real-Time Analysis (RTA), performs image analysis and base calling, and assigns a quality score to each base for each cycle. Analysis is performed during the chemistry and imaging cycles of a sequencing run. This saves downstream analysis time and allows you to quickly decide whether your run is progressing as expected.

Real-Time Analysis (RTA) runs automatically on the instrument computer, and is configured through the HCS interface at the beginning of a run.

NOTE

If for any reason RTA stops functioning, the software automatically resumes analysis during the next cycle at the appropriate point on the flow cell. Do not restart the application manually.

Data Transfer

The output from a sequencing run is a set of quality-scored base call (*.bcl) files, which are generated from the raw image files and contain the base calls per cycle. By default, images are deleted from the instrument computer after image analysis. The raw image data are not needed for downstream analysis.

If for any reason image analysis falls too far behind, the software stops sequencing and places the flow cell in a safe state. Sequencing resumes when image analysis issues are resolved.

Basecalling_Netcopy_complete

Data transfer is complete when the Basecalling_Netcopy_complete files are present in the root level of the run folder. There should be one generic file named Basecalling_Netcopy_complete.txt, plus one file for each read.

Monitor Runtime Statistics

Real-Time Analysis automatically generates quality metrics when image analysis begins. Not all metrics are available at the early cycles because some processes need multiple cycles to generate data. The following summary describes points in the run when metrics are available:

- **Image Analysis**—Begins after cycle 4. The software first needs to generate a template with cluster locations. At cycle 4, the software catches up with the image analysis.

NOTE

Because the software catches up with the image analysis during cycle 4, cycle 4 takes significantly longer to finish. The software waits for the analysis to end before proceeding with the next cycle. This behavior is normal.

- **Phasing Estimates and Base Calls**—The software generates phasing/prephasing estimates and base calls after cycle 12.
- **Quality Scores**—The software generates quality scoring after cycle 25. The software determines which of the reads pass the quality filter, applying the Chastity formula over the first 25 bases. At least 24 of the first 25 cycles must have a Chastity value >0.6. Reads may pass the quality filter if only one base in the first 25 has a lower Chastity value. Quality scores are reported only for reads passing the quality filter. See RTA Theory of Operations for more details.
- **Error Rates**—If a PhiX control was included, RTA generates error rates after cycle 25.
- **In-Line Controls**—If you prepared your samples with the TruSeq sample prep kits, RTA will perform in-line control alignment at cycle 52 of each read or the end of the read if the read length is fewer than 52 cycles.
- **Index Count**—After the last index read is completed, RTA will report the index count per lane if a sample sheet was provided.

Sequencing Analysis Viewer

The Sequencing Analysis Viewer (SAV) is an application that allows you to view important sequencing metrics generated by Real-Time Analysis (RTA) in plots, graphs, and tables. SAV automatically opens to the analysis page after image analysis begins.

Statistics are updated as soon as new analysis information is available. Select **Refresh** at any time during the run to view updated metrics as the run progresses.

SAV can be viewed from a remote location by installing SAV on another computer with access to the network location of the output run folder. You can download a copy of SAV from the Illumina website. A MyIllumina login is required.

For a detailed description of available metrics and installation instructions, see the *Sequencing Analysis Viewer User Guide*, part # 15020619.

Analysis Page

The analysis page reports the progress of the analysis, and provides access to the most important runtime statistics.

Statistics are updated as soon as new analysis information is available. The runtime statistics can be viewed from any computer with access to the experiment run folder. Navigate to the <RunFolder>\Data folder and open the Status file. The Status file is transferred to the Data subfolder in the analysis folder at the end of each cycle that is processed.

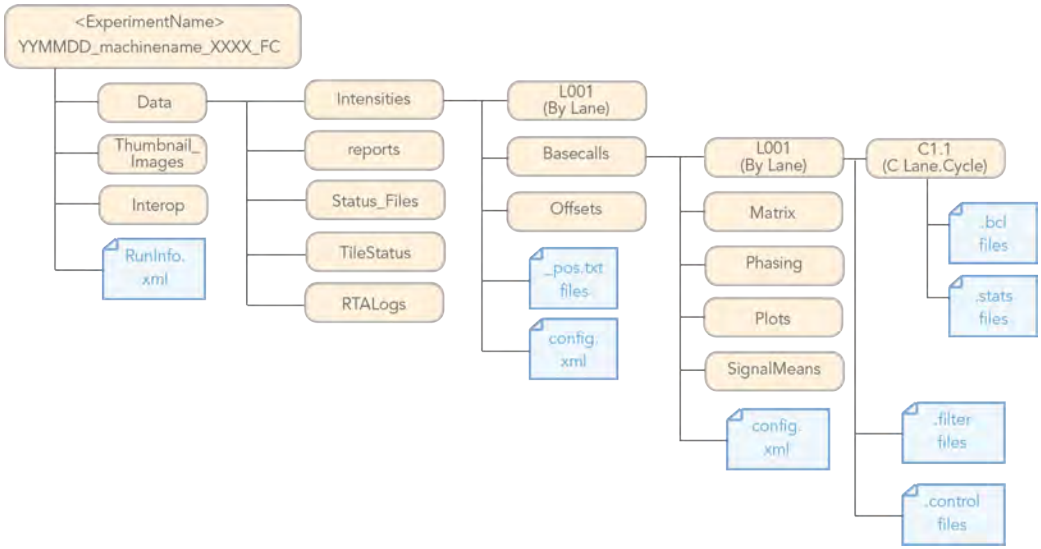
NOTE
Your browser might warn you about active content. If so, click the warning in the information bar and allow blocked content.

Real-Time Analysis Output Data

RTA output results are saved to the network location that you specified during the run setup steps.

All analysis results are saved in an analysis output folder that is given the same name as the run folder on the instrument computer. The following figure illustrates the organization of the network analysis folder, including the location of the most important output files (blue boxes).

Figure 28 Real-Time Analysis Folder



First Base Report

The First Base Report confirmation dialog box opens automatically after cycle one is complete, if you selected **Confirm First Base** on the scan screen during run setup. The run pauses at this step.

If you did not select the checkbox, the run continues after first base incorporation without prompting you to review the First Base Report.

1 Review the First Base Report from the First Base Report confirmation dialog box or by opening First_Base_Report.htm from the root level of the run folder.

2 If the results are satisfactory, select **Continue** to continue your run. If results are not satisfactory, abort the run.

Run Folders

Each sequencing run generates a run folder that contains data files and log files.

Run Folder Name and Path

During run setup, the software prompts you to enter the path for the run folder. By default, the folder is named in the following format:

YYMMDD_<Workstation Name>_<Run Number>_<Flow Cell ID>

Example: 110114_SN106_0716_A90095ACXX

The run number increments by one each time you perform a run on a given instrument. The flow cell ID entered during the run setup steps appends to the end of the run folder name.

The run folder is written to the output path specified on the Scan screen during run setup. The temporary run folder for flow cell A is written to the D: drive and the temporary run folder for flow cell B is written to the E: drive.

Contents of Run Folders

Subfolder	Key Files/Folders	Description
[Root level]	First_Base_Report.htm	File with basic metrics for the first cycle.
	IALog.txt	Log file for image analysis events.
	Sample Sheet (optional)	If you plan to use CASAVA, copy the sample sheet to the root level of the run folder. For more information, see the *CASAVA User Guide*, part # 15011196.
	RunInfo.xml	Identifies high-level run information.
	runParameters.xml	Provides the setup parameters for the run.
Config		Contains a copy of the software configuration parameters used for the run.

Subfolder	Key Files/Folders	Description
Data	Status.htm	Contains run and analysis-specific metrics generated during the run.
	Intensities folder	Contains image analysis results. This folder is saved on the network folder under <Run Folder>\Data, and is given the same name as the run folder on the instrument computer.
	CopyLog.txt	Log file for copy events.
	Log.txt	Log file for image analysis and base calling.
Data\ Intensities	BaseCalls folder	Contains RTA base calling results.
	config.xml	Contains run and image analysis-specific information.
	RTAConfiguration.xml	Configuration file for RTA in *.xml format.
Logs	Recipe_<date>_ <time>.log	Contains the run log information. Whenever the log file becomes too large (approximately 5 MB), HCS starts a new log file.
Recipe	*.xml	When you start a run, a copy of the recipe is moved to the Recipe folder. If you stop the run and resume it later, this recipe restarts where the run was stopped.
Thumbnail_ Images	thumbnail images (*.jpg files)	Contains thumbnails of nine locations from each tile, which can be used to troubleshoot a run.

Analysis Results

Table 4 Contents of Network Analysis Folder

Key File	Subfolder	Description
*.bcl files	AnalysisFolder\Data\ Intensities\Basecalls\ L00X\CX.X	Each *.bcl file contains RTA base calling and base quality scoring results for one cycle, one tile. These files are the input into CASAVA v1.8.2 or the BCL Converter.
*.cif files	AnalysisFolder\Data\ Intensities\L00X\CX.X	Each binary *.cif file contains RTA image analysis results for one cycle, one tile. Use these files in the OLB analysis software if you want to perform BUSTARD base calling. For a description, see the *Off-line Basecaller Software User Guide*.

Key File	Subfolder	Description
.clocs/.locs files	AnalysisFolder\Data\Intensities\L00	These files contain the x, y position for every cluster. The *.clocs file is a compressed version of the *.locs file.
*.filter files	AnalysisFolder\Data\ Intensities\Basecalls	*.filter files contain filter results per tile. These files are used by CASAVA v1.8.2, or later.
*.jpg files	AnalysisFolder\ Thumbnail_Images\ L00X\CX.X	HCS can generate thumbnail images of nine locations from each tile. The thumbnails are generated for each cycle and base, and can be used to troubleshoot a run.
*.stats files	AnalysisFolder\Data\ Intensities\Basecalls\ L00X\CX.X	*.stats files contain RTA base calling statistics for a given cycle/tile. These files are used by CASAVA v1.8.2 or later.
config.xml	AnalysisFolder\Data\ Intensities\ AnalysisFolder\Data\ Intensities\Basecalls\	Contains meta-information about the run, image analysis, and base calling.
CopyLog.txt	AnalysisFolder\Data	All copy events during analysis are captured in the CopyLog file.
ErrorLog.txt	AnalysisFolder\Data	Any error during analysis is captured in the ErrorLog file. If there are no errors, no ErrorLog.txt file is generated.
RunInfo.xml	AnalysisFolder\	Identifies the boundaries of the reads (including indexing reads), the flow cell ID (if available), and the quality table selected for run.
SampleSheet.csv	AnalysisFolder\ AnalysisFolder\Data\ Intensities\Basecalls\	Contains sample information, including indexing information. RTA copies the Sample Sheet file in the root folder of the run to the Basecalls folder.

Tile Numbering

The tile name is a four-digit number encoding the position on the flow cell.

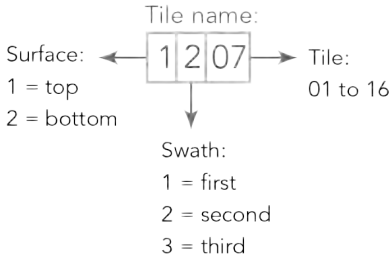
- The first digit encodes surface:
  - 1 is for top
  - 2 is for bottom
- The second digit encodes the swath:

- 1 is for the first swath
- 2 is for the second swath
- 3 is for the third swath (if applicable)

NOTE
A swath is a column of tiles within a lane of the flow cell.

- The last two digits encode the tile, 01 through 16 (with HCS v2.0). Tile numbering starts with 01 at the output end of the flow cell through 16 at the input end.

Figure 29 Tile Numbering



This example indicates a tile from the top surface of the flow cell, the second swath, and the seventh tile.

Main Sequence Output Files

The following output file types contain primary analysis data generated by Real-Time Analysis:

Base Call Files

A base call (*.bcl) file contains base calls and quality scores for one cycle and one tile. They can be used for later analysis off-instrument.

NOTE
If you are using CASAVA v1.7 or earlier, the *.bcl files need to be converted to the _qseq.txt format in OLB or the BCL Converter to enable downstream analysis. For more information, see the *BCL Converter User Guide* or the *Off-line Basecaller Software User Guide*.
This step is not necessary with CASAVA v1.8.2, or later.

Stats Files

The Stats file is a binary file containing base calling statistics for clusters passing filter. These files are used by the BCL Converter and CASAVA v1.8.

Filter Files

The filter (*.filter) files are binary files containing filter results. These files are used by the BCL Converter and CASAVA v1.8.

Control Files

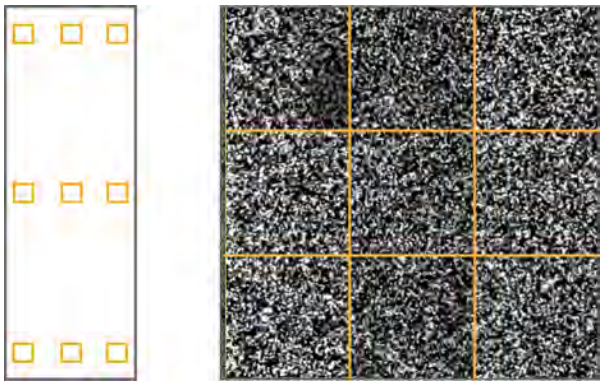
The control (*.control) files are binary files containing control results. These files are used by the BCL Converter and CASAVA v1.8.

Thumbnail Images

You can configure HCS to generate thumbnail images in *.jpg format. Thumbnail images are generated for each cycle and base.

HCS collects images from nine sections of a tile. These nine images are combined in one thumbnail image and can be used to troubleshoot a run. Thumbnail images are not suitable for image analysis.

Figure 30 Thumbnail Image



Troubleshooting

Chapter 5

Introduction 84
Possible Run Setup Problems 85
Perform a Fluidics Check 86
Repeat Read 2 Primer Hybridization 87
If BaseSpace is Unavailable 88
Stop and Resume a Run 89
Pause a Run 93

Introduction

This section describes what you can do if you encounter a problem during a sequencing run and how to perform a fluidics check from the Welcome screen.

Possible Run Setup Problems

Problem	Possible Cause	Action
The software did not initialize.	The software was unable to initialize internal hardware devices.	Close the error message and then relaunch the instrument software. If the problem persists, restart the instrument computer. If you are going to restart the computer, first shut down the instrument to make sure the DoNotEject drive is recognized correctly. If the problem persists after restarting the instrument computer, shut down the instrument, wait a minimum of 60 seconds, and then restart the instrument.
Flow cell lever is orange.	The flow cell did not seat properly. The vacuum did not seal. Manifolds did not raise.	Remove the flow cell and repeat the cleaning steps. Make sure that the gaskets are present and well-seated. Reload the flow cell.
Poor fluid delivery.	Potential bubbles in the system.	Reposition the flow cell and confirm that the holes are facing *down*. Look for white precipitate around the gaskets. If precipitate is present, replace the gaskets. Always replace gaskets before an instrument maintenance wash. Confirm that the sipper assemblies are fully lowered and each sipper is in contact with the reagents.

Perform a Fluidics Check

The Check button on the Welcome screen allows you to perform a fluidics check when you are not setting up a run. Use this option during instrument installation and fluidics troubleshooting.

1. Load a used flow cell onto the instrument.
2. Load eight 250 ml bottles with wash solution or laboratory-grade water, and load the bottles onto the corresponding reagent rack. Load the rack onto the instrument.
3. Select **Check** on the Welcome screen.
4. Select solution 5 (SB2) from the drop-down list. If you are performing a fluidics check with a used flow cell installed, you can select solution 2, which is water.
5. Enter the following default values:
   - Volume: **250**
   - Aspirate Rate: **250**
   - Dispense Rate: **2000**
6. Select **Pump**. If you need to pause the fluidics check, select **Pause**.
7. Visually inspect the flow cell for bubbles passing through the lanes and leaks near the manifolds.
   If you see excessive bubbles, check the manifold gaskets for obstructions, reduce the aspirate rate to 100, and pump another 250 µl of water to the flow cell.

Repeat Read 2 Primer Hybridization

It is possible to repeat Read 2 primer hybridization on the instrument; however, additional reagents are required. Contact Illumina Technical Support for further evaluation on whether a Read 2 rehybridization is appropriate.

For more information, visit the HiSeq support page on the Illumina website, and click **Troubleshooting**. A MyIllumina login is required.

If BaseSpace is Unavailable

If BaseSpace is not available, open Windows Services and make sure Illumina BaseSpace Broker is started; if it is not started, restart it. If services are running and you are still unable to access BaseSpace, contact Illumina technical support.

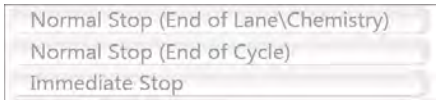
Stop and Resume a Run

You can stop a run from the Run Overview screen. You might stop a run if the run was set up incorrectly, if the data quality is bad, or if there is a hardware error. If you plan to resume your run, make sure that you select the appropriate normal stop options with one of the RTA options that allow the run resume.

Stop Option	RTA Option	Able to Resume?
Normal Stop (End of Lane\Chemistry)	Keep As Is	Yes. The run resumes at the next chemistry or imaging command.
	Complete For Run	No. The run cannot be resumed.
	Complete For Read	Yes. The run resumes at the beginning of the next read.
Normal Stop (End of Cycle)	Keep As Is	Yes. The run resumes at the next cycle.
	Complete For Run	No. The run cannot be resumed.
	Complete For Read	Yes. The run resumes at the beginning of the next read.
Immediate Stop	No option	No.

1 From the Run Overview screen, select **Stop**. The stop menu opens.

Figure 31 Stop the Run



2 Select one of the following stop options:

- **Normal Stop (End of Lane\Chemistry)**—Stops the run only after the current chemistry or imaging command is complete, and then places the flow cell in a safe state.
- **Normal Stop (End of Cycle)**—Stops the run after the current cycle is complete, and then places the flow cell in a safe state.
- **Immediate Stop**—Stops the run without completing the current operation and does ***not*** place the flow cell in safe state. You cannot resume a run that was stopped using the immediate stop option.

3 Select one of the following RTA options:

- **Keep As Is**—The run is stopped without any modifications to RTA. You can resume the run where you left off.

- **Complete For Run**—RTA is stopped. The run info, run parameters, and recipe files are updated to reflect total cycles as the last cycle completed. Then RTA restarts to complete analysis for the run up to the point the run was stopped. You cannot resume the run.
- **Complete For Read**—RTA is stopped. The run info, run parameters, and recipe files are updated to trim the length of the current read to the last cycle completed. Subsequent reads are not affected. Then RTA restarts to complete analysis for the current read. You can resume the run at the beginning of the next read.

4 After the run is stopped, select **Return to Start** on the Run Overview screen. The Welcome screen opens.

Resume a Stopped Run

You can resume a stopped run if the run was stopped safely using a normal stop option with one of the RTA options that allow the run to resume.

1 From the Welcome screen, select **Sequence**, and then select **Resume Run**. The Resume screen opens.

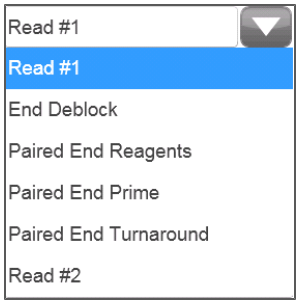
2 Select the run folder for the run you want to resume from the drop-down list.

NOTE
The software resumes the run at the point where the run was stopped, and defaults to the correct setup on the Resume screen.

3 Confirm the settings on the Resume screen or select the appropriate point in the run to resume the run.

- **Resume At** lists the read or point in the run to resume.
- **Start At Cycle** lists the cycle to resume.

Figure 32 Points to Resume the Run



CAUTION
Illumina does not recommend resuming a run at the point of paired-end turnaround, other than for Read 2 primer rehybridization. For more information, see *Repeat Read 2 Primer Hybridization* on page 87.

4 Confirm the settings on the Resume screen or select the imaging and chemistry commands to resume. For more information, see *Example Settings for Resuming a Run* on page 91.

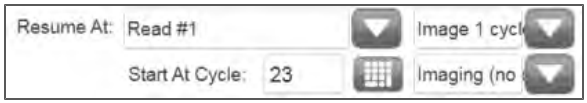
5 Select **Next** to proceed. The software guides you through the remaining run setup steps.

Example Settings for Resuming a Run

If you stopped a run after imaging lane 1 at cycle 23 of a 101-cycle read, the software automatically sets up the resume run settings for Read #1 at cycle 23. The system displays the following settings on the Resume screen:

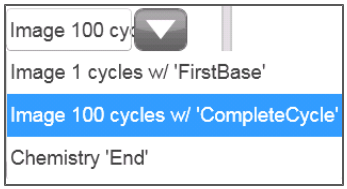
- **Resume At:** Read #1
- **Start At Cycle:** 23

Figure 33 Example of Resume at Cycle 23



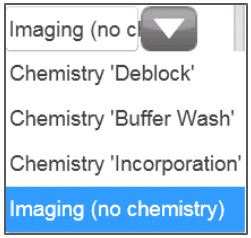
For a 101-cycle read, there is one cycle of **FirstBase** followed by 100 cycles of **CompleteCycle**. At cycle 23, you are within 100 cycles of CompleteCycle. **Image 100 cycles w/ 'CompleteCycle'** is selected automatically.

Figure 34 Examples of Imaging Commands



Because the run in this example was stopped during an imaging step, **Imaging (no chemistry)** is selected automatically.

Figure 35 Examples of Chemistry Commands



Pause a Run

You can pause a run from the Run Overview screen. You might pause a run to check run components, such as reagent volumes, before proceeding with the run. Under normal procedures, you do not need to use this feature. The software pauses the run automatically before Read 2 to allow you to load reagents.

1 From the Run Overview screen, select **Pause**. The pause menu opens.

Figure 36 Pause Options

2 Select **Normal Pause**.

3 Select **Yes** to confirm the pause command. The software completes the current chemistry or imaging command and places the flow cell in a safe state.

4 Select **Resume** when you are ready to resume the run.

Change Reagents During a Run

If you started your run with a partial volume of reagents, use the Change Reagents feature to pause your run and replenish reagents. There is no need to pause a run to load fresh reagent before Read 2; the software pauses the run automatically at that point.

1 From the Run Overview screen, select **Pause**. The pause menu opens.

2 Select **Change Reagents**.

3 Select **Yes** to confirm the pause command. The software completes the current chemistry or imaging command and places the flow cell in a safe state.

4 On the Reagent screen, enter the following reagent parameters:
- The reagent kit ID for new reagents you are loading.
- The number of cycles the reagents are expected to last.

NOTE
The priming checkbox is disabled on the Change Reagents screen. Priming is not required.

5 Select **Next** to proceed to loading reagents.

Index

A

activity indicators 22
analysis folder 77
analysis page 75-76
analysis results 79, 81

B

base calling results 78
bcl files 79, 81

C

cif files 79
clicking sound 56
clocs files 80
components
    flow cell compartment 6, 8, 43
    fluidics compartment 6
    optics module 6
    reagent compartment 6-7, 63
config.xml 78, 80
consumables
    Illumina sequencing kits 16
    user supplied 17
control files 81
customer support 99

D

data transfer 74
disk space, checking 15
documentation 99
dual-indexed libraries 13
dual indexing
    on paired-end flow cell 35
    overview 11
    settings on recipe screen 35

F

filter files 80-81
first base incorporation
    confirmation 34, 57, 77
    report location 34, 78
flow 51
flow cell
    cleaning 52
    flow cell ID 31, 34
    lever 45, 53
    loading 43, 50
    troubleshooting 85
flow cell compartment 6, 8, 43
Flow Cell Set-up Screen 34
fluidics check 86
fluidics compartment 6
fluidics, troubleshooting 85

H

help, technical 99
HiSeq Control Software 15, 19

I

icons
    errors and warnings 23
    status alert 23
idling the instrument 70
images
    saving images samples 32
    thumbnails 32
indexed sequencing
    reagents, loading 42
indexing
    reagents 30
initialize software 20
instrument wash 62, 68

L

loading reagents 40, 59, 63
locs files 80

M

maintenance wash 62
monitoring 56
  cluster intensities 56
  image samples 56
  run fluidics 56

N

normal stop 89

O

optics module 6

P

paired-end sequencing
  reagents, loading 59
post-run procedures 62
primer rehybridization 87
priming
  preparation 48
  waste volume 48

Q

quality scores
  generating 75

R

Read 2 hybridization 87
reagent compartment 6-7, 63
reagent positions
  indexing 42
  paired end 59
reagents
  loading 42, 59, 63
  loading, ICB 60
  loading, SBS 40
  paired end 59
  priming 48
  Reagents screen 37
  recording kit ID 37
  sequencing 63
Reagents screen 37
Real Time Analysis 19, 74
  analysis folder 77
  analysis page 75
  analysis results 79
  data transfer 74
  runtime statistics 75
  status page 76
  with stopped run 89
recipe folder 78
recipe screen 31
reports
  first base incorporation 34, 57, 77-78
  run folder contents 78
resume run 21
review run setup screen 31
run folders 78
run overview screen 56
RunInfo.xml 78, 80

S

sample sheet
  in run folder 78
  overview 14
SAV 57, 75
scan screen 31
Sequencing Analysis Viewer 19, 57, 75-76
sequencing consumables 16
show log file 20
single indexing
  overview 9
software
  about, version 20
  Experiment Manager 14
  HiSeq Control 15
  HiSeq Control Software 19
  initialization 18
  initialize manually 20
  Real Time Analysis 19
  RTA 74
  Sequencing Analysis Viewer 19
  show log file 20
  troubleshooting 85
  view full screen 20
sound, clicking 56
stats files 80-81
status alert icon 23
status page 56
status.xml 78
stopping a run 89

Storage
    BaseSpace 32
Storage screen 32
swath 32

T

technical assistance 99
tile numbering 80
troubleshooting 85
turning off instrument 71

U

user-supplied consumables 17

W

washes
    consumables 68
    delivered volumes 68
    maintenance wash 21, 62
    recommendations 62
    water wash 21, 62, 68
water wash 62

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 5 Illumina General Contact Information

Illumina Website	www.illumina.com
Email	techsupport@illumina.com

Table 6 Illumina Customer Support Telephone Numbers

Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Italy	800.874909
Austria	0800.296575	Netherlands	0800.0223859
Belgium	0800.81102	Norway	800.16836
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000

MSDSs

Material safety data sheets (MSDSs) are available on the Illumina website at www.illumina.com/msds.

Product Documentation

Additional product documentation in PDF is available for download from the Illumina website. Go to www.illumina.com/support and select a product. A MyIllumina login is required. To register for a MyIllumina account, please visit my.illumina.com/Account/Register.